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STUDIES ON HOG CHOLERA.*

EXPERIMENTAL HYPERIMMUNIZATION.

WALTER E. KING AND ROBERT H. WILSON.

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During the period that *B. choleræ suis* was accepted as the causative factor of hog cholera, investigators attempted to attenuate the organism sufficiently to produce a practical vaccine. Chemicals, heat, and various other methods were employed for this purpose, but the results obtained with these vaccines were not entirely satisfactory. In some instances they protected hogs inoculated with cultures of *B. choleræ suis,* but in the field, as a rule, failed to protect against natural infection.

When it became known that the specific cause of cholera was involved in the so-called "filterable virus," attention was turned to its modification for preventive inoculation. Practically all of the experimental methods used in the attenuation of *B. choleræ suis* have been unsuccessfully applied to the virus. Numerous experiments have been conducted in attempting to attenuate the virus by heating. Dorset and Niles[1] have worked along this line with negative results. Graham[2] also reports unfavorable results with similar experiments. Peters[3] claims to have had more or less success with serum virus heated at a temperature of 60° C. for one-half hour. He has inoculated over 16,000 hogs with this attenuated virus and reports good results in many herds. It is the consensus of opinion, however, that this method of attenuation is not reliable for field use, owing to the difficulty in obtaining a uniform product, and the fact that there is a great variation in the susceptibility of hogs to cholera.

In 1908, King[4] published results from the attempted attenuation of hog cholera virus by passage through animals of other

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*Received for publication October 1, 1912.

[1] U. S. Live Stock Sanitary Proceedings, 1911.
[2] *Amer. Vet. Rev.*, 1912.
[3] U. S. Live Stock Proceedings, 1911.
[4] Bull. Kansas Exp. Sta., 1908, No. 157.

species, particularly the horse. He concluded that the virus undergoes some unexplainable modification resulting in an attenuation after several hours' residence in the circulatory system of the horse. He noted that during the first two hours' residence the virus seemed to become activated in some manner. Typical cases of acute cholera were produced in normal hogs by the injection of two to four cubic centimeters of serum drawn from a horse which had received intravenously a quantity of hog cholera virus two hours previously. Serum drawn at a later period than this seemed to be less virulent, the virulence decreasing in proportion to the length of time elapsing after the horse had received the cholera virus.

In 1910, we[1] attempted to determine whether or not "horse serum virus" (serum drawn from a horse within two hours after the injection of hog cholera virus) represents a mere dilution of the infectious agent. Tests were made to determine this point by preparing corresponding dilutions of the same strains of virus in normal horse blood *in vitro* and also in sterile physiologic salt solution. The proportionate dilutions were made by weighing the horse and taking one-fifteenth of the body weight as representing the total amount of blood in the animal. These dilutions were then kept at body temperature for the same period of time as that of the residence of the cholera virus in the circulatory system of the horse. The virulence of each dilution was tested by the injection of normal hogs kept in separate, well isolated pens. As noted, the serum drawn from the horse within two hours after injection with virus produced cases of acute cholera with an average incubation period of seven days, and an average duration of the disease of 15 days. With the same lot of virus in proportionate dilution in normal horse blood and physiologic salt solution *in vitro,* fewer cases of cholera were produced and those which did develop had a longer average incubation period, and were more chronic in nature. These results indicated that horse serum virus does not represent a mere dilution of cholera virus.

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[1] Ibid., 1910, No. 171.

HYPERIMMUNIZATION WITH HORSE SERUM VIRUS.

I. EXPERIMENTAL.

The fact that serum drawn from a horse one or two hours after it receives an intravenous injection of approximately 150 Cc. of cholera virus, is capable of producing acute cholera when injected in small doses into normal hogs, suggested the possibility of substituting horse serum virus for hog serum virus in the production of Dorset-Niles hyperimmune serum. Assuming that a reliable, potent serum could be produced by this modified method certain advantages would follow:

The cost of production would be less than that involved in the present method. The serum from one horse could be substituted for the blood from a large number of hogs. This would result, not only in greater economy, but in greater convenience and in better regulated manipulations in serum production. Furthermore, more adequate precautions against contamination could be exercised.

We have published the results of some preliminary experiments relative to hyperimmunization with horse serum virus. These results were fairly uniform in character and indicated that the procedure warranted further experimentation. During the past two years the following data have been collected on this subject.

The virus used in the injection of the horses was in every instance secured from cases of acute cholera in which the symptoms and lesions were characteristic. The moribund hogs were bled from the carotid artery under aseptic conditions. The receptacle containing the blood was placed in a refrigerator for 10 or 12 hours to allow the coagulum to harden and contract. The serum was then poured into sterile flasks which were placed in the refrigerator pending the time of injection.

The horses used in the work, three in number, weighed approximately 1100 pounds each. They were each about nine years old and constitutionally sound. Each was injected and bled four times. The amount of hog cholera serum injected into the horses at a given time varied from 100 to 150 Cc. The injections, in practically every case, were followed by severe reactions, beginning five or ten minutes after the horse had received the virus. The chief characteristics of these reactions were increased respiratory and cardiac movements, stimulation of nearly all the body secretions, and marked general depression. The first symptoms usually disappeared in one hour, leaving the horse more or less exhausted for a day or two. An important feature noted in connection with the injection was that the reactions were usually more pronounced after the first injection. A horse may be repeatedly treated with hog cholera virus intravenously for an indefinite period, as the three animals used in this work are at present alive and in good condition. The bleedings were made one-half to three-quarters of an hour after injection, experience showing that serum drawn from the horse at this time is more virulent than that drawn at an earlier or later period. A specially devised apparatus which defibrinates the blood as it is drawn was used in bleeding. About six liters of defibrinated blood were obtained at each bleeding.

TABLE 1.

VIRULENCY TESTS OF HORSE SERUM.

Hog	Weight	Date	Amount Serum	Horse	Results	
175.......	46	9/23	4 c.c.	1,313	Symptoms after 3 days; died after 15 days	Typical lesions
196.......	55	11/15	4 "	1,313	Symptoms after 5 days; died after 19 days	Typical lesions
250.......	55	2/28	4 "	1,422	Symptoms after 8 days; died after 30 days	Typical lesions
251...	48	2/28	4 "	1,422	Symptoms after 10 days; died after 27 days	Typical lesions
278.......	60	4/22	4 "	1,423	Symptoms after 6 days; died after 20 days	Typical lesions
279.....	56	4/22	4 "	1,422	Symptoms after 3 days; died after 13 days	Typical lesions
292......	80	5/17	4 "	1,423	Symptoms after 5 days; died after 14 days	Typical lesions
293.......	80	5/20	4 "	1,314	Symptoms after 7 days; died after 14 days	Typical lesions
294......	75	5/22	4 "	1,314	Symptoms after 5 days; died after 15 days	Typical lesions
295.......	95	5/29	4 "	1,422	No reaction	(Natural immune?)
297.....	95	6/9	4 "	1,422	Symptoms after 5 days; died after 12 days	Typical lesions
301......	48	6/26	4 "	1,423	Symptoms after 4 days; died after 22 days	Typical lesions
334.....	40	12/4	4 "	1,422	Symptoms after 12 days; died after 22 days	Typical lesions
345.....	50	11/8	4 "	1,422	Symptoms after 6 days; died after 27 days	Typical lesions

The horse serum virus was tested for virulency before being used for experimental hyperimmunization. One or more normal hogs were injected, subcutaneously, each with four cubic centimeters of the serum from the horse under test. These test hogs were kept in well isolated pens and every precaution was observed to guard against extraneous infection. Table I shows the results of these tests. It will be noted that of the 14 cases reported in the table only one failed to contract cholera from the horse serum virus. Three or four of the animals developed chronic cases, but all succumbed to the disease, showing typical lesions. The average incubation period was 6.2 days and the average duration of the disease 20 days.

Thirteen immune hogs received injections of horse serum virus for the purpose of experimental hyperimmunization. The technique employed in the injecting, bleeding, and handling of the serum varied but little from that in use at most of the hog cholera serum laboratories. The intraperitoneal method of injection was used in practically all cases. One-half of one per cent carbolic acid was added to the defibrinated blood as a preservative. Table 2 gives the number of experimental hyperimmune hogs, their weight, method of injection, amount of defibrinated blood injected, and the number of bleedings.

TABLE 2.

EXPERIMENTAL HYPERIMMUNES.

Hog	Weight	First Injection	Injection Amount	Bleedings	Second Injection	Injection Amount	Bleedings
173	200	Intraperitoneally and intravenously	1,700 c.c	8	Intraperitoneally	1,600 c.c.	4
174	175	Intraperitoneally	900 "	8	.....	....	..
177	230	"	1,600 "	8	Intraperitoneally	1,800 c.c.	3
217	58	"	1,400 "	..	"	800 "	7
221	300	"	2,400 "	3	"	2,200 "	11
254	200	"	2,000 "	..	"	1,000 "	13
255	190	"	1,800 "	..	"	900 "	14
272*A*	140	"	1,460 "	10	.....	....	..
288	190	"	1,800 "	..	.....	900 c.c.	11
289	196	"	2,000 "	Died	.....	....	..
290	185	"	1,800 "	Died	.....	....	..
291	190	"	1,800 "	..	Intraperitoneally	1,200 c.c.	15
296	100	"	900 "	10	.....	....	..

Hyperimmune 173.—A Poland China, weight 200 pounds, immunized by the injection of 70 Cc. hyperimmune serum (Mich. Agr. College) and 2 Cc. of virus on September 13, 1910. Fourteen days later it was injected with 1700 Cc. of ¾-hour horse serum from horse 1314. Of this amount, 520 Cc. were given intravenously and the remainder intraperitoneally. The hog experienced considerable difficulty in breathing and was otherwise depressed for a few hours after receiving the serum. The first bleeding was made on October 20, when 400 Cc. of serum was obtained. The bleedings were continued at intervals of a week until eight bleedings had been made. As the serum from these various bleedings did not protect the

test hogs against cholera, it was deemed advisable to give the hog another injection of the horse serum virus to determine whether this would increase the potency of its serum. This second injection consisted of 1600 Cc. of horse serum virus given intraperitoneally on February 16, 1911. The next tail bleeding was made on March 21, followed by three others at intervals of one week. The hog was bled to death on April 19, 1800 Cc.

TABLE 3.

POTENCY TESTS OF SERUM FROM HOG 173.

Bleeding	Hog	Weight	Date	Material Injected	Results	
1st.	183	56	10/20	25 c.c. serum + 2 c.c. virus	Symptoms after 11 days; died after 32 days	Cholera lesions not pron'ced
	184	59	10/20	35 c.c. serum + 2 c.c. virus	Symptoms after 11 days; died after 23 days	Fair cholera lesions
	185	51	10/20	2 c.c virus	Symptoms after 5 days; died after 13 days	Typical lesions
2d	191	52	10/27	25 c.c. serum + 2 c.c. virus	Symptoms after 18 days; died after 21 days	Typical lesions
3d	191	58	10/27	35 c.c. serum + 2 c.c. virus	Symptoms after 7 days; died after 17 days	Typical lesions
	192	53	10/27	35 c.c. serum + 2 c.c. virus	Symptoms after 10 days; died after 18 days	Typical lesions
	193	65	10/27	2 c.c. virus	Symptoms after 4 days; died after 8 days	Typical lesions
4th	195	53	11/14	25 c.c. serum + 2 c.c. virus	Remained well	
	210	50	11/14	4.6 gm. dried serum + 2 c.c. virus	Symptoms after 7 days; died after 19 days	Typical lesions
	211	55	11/14	6.4 gm. dried serum + 2 c.c. virus	Symptoms after 7 days; died after 21 days	Typical lesions
	212	46	11/14	2 c.c. virus	Symptoms after 6 days; died after 8 days	Typical lesions
6th	228	31	1/25	25 c.c. serum + 1.5 c.c. virus	Symptoms after 12 days; died after 18 days	Typical lesions
	229	40	1/25	35 c.c. serum + 1.5 c.c. virus	Symptoms after 11 days; died after 16 days	Typical lesions
	230	33	1/25	1.5 c.c. virus	Symptoms after 6 days; died after 11 days	Typical lesions
7th	235	50	2/3	35 c.c. serum + 2 c.c. virus	Symptoms after 9 days; died after 18 days	Typical lesions
	236	34	2/3	25 c.c. serum + 2 c.c. virus	Symptoms after 6 days; died after 12 days	Typical lesions
	233	38	2/3	2 c.c. virus	Symptoms after 5 days; died after 18 days	Typical lesions
8th	247	40	2/22	25 c.c. serum + 2 c.c. virus	Symptoms after 4 days; died after 23 days	Fair lesions
1st after 2d injection horse serum	256	50	3/21	50 c.c. serum + 2 c.c. virus	Remained well	
	257	50	3/21	40 c.c. serum + 2 c.c. virus	Died after 19 days	Not cholera
	259	48	3/21	2 c.c. virus	Symptoms after 5 days; died after 9 days	Fair lesions
3d	271	100	4/10	40 c.c. serum + 2 c.c. virus	Remained well	
	273	40	4/10	2 c.c. virus	Symptoms after 6 days; died after 15 days	Typical lesions
Slaughter . .	283	90	4/28	40 c.c. serum + 2 c.c. virus	Remained well	
	284	88	4/28	30 c.c. serum + 2 c.c. virus	Symptoms after 12 days; died after 30 days	Fair lesions
	286	67	4/28	2 c.c. virus	Symptoms after 7 days; died after 11 days	Typical lesions

of blood being obtained. Postmortem examination of the hog revealed no pathological conditions resulting from the injection of horse serum virus.

The serum from hog 173 in most instances failed to protect the test hogs from cholera. It will be noted, however, that the incubation period and duration of the disease were considerably longer in the serum test hogs than in the controls, indicating that a slight resistance to cholera resulted from the injection of the serum.

Hyperimmune 174.—Yorkshire sow, weight 175 pounds. Immunized by the injection of 70 Cc. hyperimmune serum (Mich. Agric. College) and 2 Cc. of cholera virus. Two weeks later injected intraperitoneally with 850 Cc. of ¾-hour horse serum virus. A slight reaction followed the injection as evidenced by anorexia and listlessness lasting a day. First bleeding on October 19, 22 days after receiving the horse serum. Eight bleedings made at intervals of a week.

TABLE 4.

POTENCY TESTS OF SERUM FROM 174.

Bleeding	Hog	Weight	Date	Material Injected	Results	
1st........	180	58	10/19	25 c.c. serum + 2 c.c. virus	Symptoms after 7 days; died after 17 days	Typical lesions
	181	65	10/19	35 c.c. serum + 2 c.c. virus	Remained well	
	182	62	10/19	2 c.c. virus	Symptoms after 10 days; died after 20 days	Typical lesions
2d........	187	85	10/26	40 c.c. serum + 2 c.c. virus	Symptoms after 20 days; died after 25 days	Typical lesions
	188	62	10/26	30 c.c. serum + 2 c.c. virus	Symptoms after 19 days; died after 24 days	Fair lesions
	189	65	10/26	2 c.c. virus	Symptoms after 5 days; died after 5 days	Typical lesions
3d........	204	45	12/19	25 c.c. serum + 2 c.c. virus	Remained well	
4th.......	205	58	12/19	25 c.c. serum + 2 c.c. virus	Remained well	
5th.......	214	50	1/12	25 c.c. serum + 2 c.c. virus	Remained well	
	215	54	1/12	35 c.c. serum + 2 c.c. virus	Remained well.	
	216	49	1/12	2 c.c. virus	Symptoms after 6 days; died after 14 days	Typical lesions
6th.......	222	75	1/24	40 c.c. serum + 2 c.c. virus	Remained well	
	223	58	1/24	25 c.c serum + 2 c.c. virus	Remained well	
	224	56	1/24	2 c.c. virus	Symptoms after 7 days; died after 12 days	Typical lesions
7th.......	237	45	2/8	25 c.c. serum + 2 c.c. virus	Remained well	
	238	59	2/8	30 c.c. serum + 2 c.c. virus	Remained well	
	239	48	2/8	2 c.c. virus	Symptoms after 6 days; died after 13 days	Typical lesions
	288	190	5/6	75 c.c. serum + 2 c.c. virus	Remained well	
	289	196	5/6	75 c.c. serum + 2 c.c. virus	Remained well	
Slaughter ..	290	185	5/12	75 c.c. serum + 2 c.c virus	Remained well	
	291	190	5/12	75 c.c. serum + 2 c.c. virus	Remained well	
	286	68	4/28	2 c.c. virus	Symptoms after 7 days; died after 11 days	Typical lesions

With the exception of the first two bleedings, the serum from hog 174 afforded ample protection to the test hogs.

Hyperimmune 177.—A Poland China, weight 230 pounds, received immunizing treatment October 4, consisting of 55 Cc. of hyperimmune serum (Mich. Agric. College) and 2 Cc. of virus. Twenty-seven days later was given intraperitoneally 1600 Cc. of ¾-hour horse serum virus. First bleeding made November 8. The serum from the first eight bleedings did not prove to be sufficiently potent for practical purposes, and the hog was given a second injection of horse serum February 21, consisting of 1600 Cc. of serum. Bleedings resumed one month later. Slaughter bleeding April 26.

TABLE 5.

POTENCY TESTS OF SERUM FROM 177.

Bleeding	Hog	Weight	Date	Material Injected	Results	
1st........	194	55	11/9	25 c.c. serum + 2 c.c. virus	Symptoms after 7 days; died after 21 days	Slight lesions
2d.........	197	49	11/16	25 c.c. serum + 2 c.c. virus	Symptoms after 7 days; died after 19 days	Typical lesions
3d........	207	46	12/23	25 c.c. serum + 2 c.c. virus	Remained well	
	208	51	12/23	35 c.c. serum + 2 c.c. virus	Symptoms after 8 days; died after 22 days	Fair lesions
	209	43	12/23	2 c.c. virus	Symptoms after 7 days; died after 17 days	Typical lesions
5th.......	217	58	1/16	35 c.c. serum + 2 c.c. virus	Remained well	
	218	50	1/16	25 c.c. serum + 2 c.c. virus	Remained well	
	219	51	1/16	2 c.c. virus	Symptoms after 5 days; died after 10 days	Typical lesions
6th.......	225	35	1/24	25 c.c. serum + 2 c.c. virus	Symptoms after 11 days; died after 23 days	Typical lesions
	226	55	1/24	35 c.c. serum + 2 c.c. virus	Remained well	
	227	38	1/24	15 c.c. virus	Symptoms after 5 days; died after 9 days	Typical lesions
7th.......	232	50	2/3	35 c.c. serum + 2 c.c. virus	Symptoms after 13 days; died after 21 days	Lesions not pronounced
	233	44	2/3	25 c.c. serum + 2 c.c. virus	Symptoms after 16 days; died after 21 days	Lesions not pronounced
	234	45	2/3	2 c.c. virus	Symptoms after 5 days; died after 18 days	Typical lesions
1st after 2d injection horse serum	258	45	3/21	25 c.c. serum + 2 c.c. virus	Symptoms after 24 days; died after 34 days	Typical lesions
	259	38	3/21	2 c.c. virus	Symptoms after 5 days; died after 9 days	Typical lesions
2d........	272	64	4/10	30 c.c. serum + 2 c.c. virus	Symptoms after 10 days; died after 28 days	Typical lesions
Slaughter..	285	52	4/28	30 c.c. serum + 2 c.c. virus	Remained well	
	286	85	4/28	40 c.c. serum + 2 c.c. virus	Remained well	
	287	67	4/28	2 c.c. virus	Symptoms after 8 days; died after 12 days	Typical lesions

These experiments indicate that the serum from 177 was lacking in protective properties. The second injection of horse serum apparently failed to increase the potency to any extent.

Hyperimmune 217.—Yorkshire, weight 58 pounds, immunized January

16, with 35 Cc. of hyperimmune serum (175, 5th bleeding) and 2 Cc. virus. On May 3, received 1400 Cc. of ¾-hour horse serum and 800 Cc. one month later, no bleedings intervening. The first bleeding was made July 3, and bleedings continued weekly until September 1, when the hog was killed.

TABLE 6.

POTENCY TESTS OF SERUM FROM 217.

Bleeding	Time After Injection	Hog	Weight	Material Injected	Results	
3, 4, 5, 6, 7, slaughter..	40,47,54,61, 68 days..	321	55	35 c.c. serum + 2 c.c. virus	Remained well	
		322	45	25 c.c. serum + 2 c.c. virus	Remained well	
		323	42	2 c.c. virus	Symptoms after 5 days; died after 18 days	Typical lesions

The blood from all the bleedings of 217 was combined and tested collectively, with the result that both hogs which received treatment were sufficiently protected, while the control succumbed to the disease.

Hyperimmune 221.—Poland China, weight 250 pounds. Immunized January 23, with 75 Cc. serum (174, 3d bleeding), and 2 Cc. virus. On February 4, was injected intraperitoneally with 2600 Cc. of ¾-hour horse

TABLE 7.

POTENCY TESTS OF SERUM FROM 221.

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
1st.........	33 days ...	253	35	25 c.c. serum + 2 c.c. virus	Remained well	
2d.........	43 days....	259	58	40 c.c. serum + 2 c.c. virus	Symptoms after 12 days; died after 23 days	Fair lesions
		260	40	30 c.c. serum + 2 c.c. virus	Symptoms after 11 days; died after 20 days	Typical lesions
		261	36	2 c.c. virus	Symptoms after 5 days; died after 9 days	Fair lesions
3d.........	....	266	75	40 c.c. serum + 2 c.c. virus	Symptoms after 14 days; died after 35 days	Typical lesions
		267	55	30 c.c. serum + 2 c.c. virus	Symptoms after 10 days; died after 30 days	Fair lesions
		268	70	2 c.c. virus	Symptoms after 6 days; died after 13 days	Typical lesions
1st after 2d injection..	30 days	296	99	40 c.c. serum + 2 c.c. virus	Remained well	
5th........	62 days	305	42	40 c.c. serum + 2 c.c. virus	Remained well	
		306	39	30 c.c. serum + 2 c.c. virus	Symptoms after 7 days; died after 26 days	Fair lesions
		307	39	2 c.c. virus	Symptoms after 6 days; died after 19 days	Typical lesions
6th........	67 days	308	135	40 c.c. serum + 2 c.c. virus	Remained well	
7, 8, 9, 10, slaughter..	74,81,88,95	314	125	40 c.c. serum + 2 c.c. virus	Remained well	
		315	50	30 c.c. serum + 2 c.c. virus	Remained well	
		317	125.	2 c.c. virus	Severe reaction, recovered	

serum. The first bleeding was made a month later, followed by two others at intervals of a week. A second injection of ¾-hour horse serum was given April 22, consisting of 2200 Cc. Bleedings were resumed one month later and continued weekly for 11 weeks.

The potency tests showed that the serum from the first three bleedings from 221 was lacking in protective properties. That the test hogs received a degree of immunity is evidenced by the fact that the incubation period and duration of the disease were more prolonged than was the case with the controls in the same tests. The second injection of horse serum stimulated the formation of additional antisubstances. It will be noted that the blood from the seventh to slaughter bleedings was mixed and a composite test made, owing to the shortage of hogs at the time.

TABLE 8.

POTENCY TESTS OF SERUM FROM 254.

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
2d.........	30 days	298	77	40 c.c. serum + 2 c.c. virus	Symptoms after 12 days; died after 22 days	Typical lesions
		299	64	25 c.c. serum + 2 c.c. virus	Symptoms after 13 days; died after 22 days	Typical lesions
		300	60	2 c.c. virus	Symptoms after 8 days; died after 22 days	Typical lesions
3d.........	44 days	309	56	40 c.c. serum + 2 c.c. virus	Symptoms after 8 days; died after 17 days	Typical lesions
		310	58	30 c.c. serum + 2 c.c. virus	Symptoms after 8 days; died after 14 days	Lesions not pron'ced
		313	46	2 c.c. virus	Symptoms after 5 days died after 12 days	Less pronounced
4, 5, 6, 7, 8, 9	51, 58, 65 72, 77, 84	319	47	35 c.c. serum + 2 c.c. virus	Remained well	
		320	37	25 c.c. serum + 2 c.c. virus	Remained well	
		322	45	2 c.c. virus	Symptoms after 5 days; died after 12 days	Lesions not pron'ced
10, 11, 12, 13, 14, slaughter	91, 98, 105, 112, 119	329	65	40 c.c. serum + 2 c.c. virus	Remained well	
		330	62	30 c.c. serum + 2 c.c. virus	Remained well	
		333	65	2 c.c. virus	Symptoms after 6 days; died after 9 days	Typical lesions

Hyperimmune [illegible].—Yorkshire sow, weight 200 pounds. Received immunizing treatment March 17, consisting of 75 Cc. serum (hog 174, 5th bleeding) and 2 Cc. virus. On April 12 received intraperitoneally 1800 Cc. of ¾-hour horse serum and 900 Cc. May 16. No ill effects followed these treatments other than a slight soreness, which soon passed off. The first bleeding was made one month after the second injection of horse serum. The hog was bled 15 times, and slaughtered September 22.

The first three bleedings yielded a serum which did not afford protection against cholera. Serum from the later bleedings was sufficiently potent to immunize the test hogs.

Hyperimmune [illegible].—Yorkshire weight 190 pounds. Immunized May 17, with 75 Cc. serum (hog 174, 5th bleeding) and 2 Cc. virus. On April 12 and May 16, injected with 1800 Cc. and 900 Cc., respectively, of ¾-hour horse serum. No pronounced reactions were caused by these injections. Bleeding commenced one month after second injection.

TABLE 9.

POTENCY TESTS OF SERUM FROM 255.

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
2d..........	30 days	302	75	40 c.c. serum + 2 c.c. virus	Remained well	
		303	30	30 c.c. serum + 2 c.c. virus	Symptoms after 8 days; died after 28 days	Lesions not pron'ced
		304	64	2 c.c. virus	Symptoms after 8 days; died after 18 days	Typical lesions
3d..........	46 days	311	62	40 c.c. serum + 2 c.c. virus	Symptoms after 10 days; died after 21 days	Typical lesions
		312	58	30 c.c. serum + 2 c.c. virus	Symptoms after 10 days; died after 19 days	Typical lesions
		313	46	2 c.c. virus	Symptoms after 6 days; died after 13 days	Lesions not pron'ced
4, 6, 7......	53, 67, 74	316	120	45 c.c. serum + 2 c.c. virus	Remained well	
		317	130	45 c.c. serum + 2 c.c. virus	Remained well	
		318	125	2 c.c. virus	Severe reaction, recovered	
8 to 15.....	60, 67, 74, 82, 89, 96, 103	331	62	40 c.c. serum + 2 c.c. virus	Remained well	
		332	55	30 c.c. serum + 2 c.c. virus	Remained well	
		333	65	2 c.c. virus	Symptoms after 6 days; died after 9 days	Typical lesions

Hyperimmune 272A.—A Poland China stag, weight 146 pounds. Vaccinated April 10, with 40 Cc. hyperimmune serum and 2 Cc. virus (hog 177, 3d bleeding). Received but one injection of horse serum (1500 Cc.), this being May 29. Was not bled until three months later.

TABLE 10.

POTENCY TESTS OF SERUM FROM 272*A*

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
1 to 10.....	1st, 63 days, others weekly	339	52	40 c.c. serum + 2 c.c. virus	Remained well	
		340	51	30 c.c. serum + 2 c.c. virus	Remained well	
		341	58	2 c.c. virus	Symptoms after 6 days; died after 16 days	Typical lesions

This experiment shows that the various lots of serum from 272A, tested collectively, protected hogs from the virus. This animal received but one treatment of horse serum and was not bled until three months later, suggesting that considerable time must elapse between the injection of horse serum and the first bleeding in order to obtain a more potent serum.

Hyperimmune 288.—Yorkshire sow, weight 190 pounds. Immunized to cholera May 6, with 75 Cc. serum (hog 174, 6th bleeding) and 2 Cc. virus. Injected twice with ¾-hour horse serum May 20, 1800 Cc., and June 22, 900 Cc. Bleedings started one month later.

Hyperimmune 291.—Poland China, weight 190 pounds. Immunized May 12, with 75 Cc. serum (hog 221, 4th bleeding) and 2 Cc. virus. In-

TABLE 11.

POTENCY TESTS OF SERUM FROM 288.

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
1 to 11.....	1st, 34 days	337	72	40 c.c. serum + 2 c.c. virus	Remained well	
	Others weekly	338	56	30 c.c. serum + 2 c.c. virus	Symptoms after 6 days; died after 16 days	Typical lesions
		341	58	2 c.c. virus	Symptoms after 6 days; died after 16 days	Typical lesions

jected May 31, with 1800 Cc. of 34-horse serum and again June 30 with 1200 Cc. No reaction followed these injections. Bleedings started July 29, and continued at intervals of a week.

TABLE 12.

POTENCY TESTS OF SERUM FROM 291.

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
1 to 7......	30 days	345	70	40 c.c. serum + 2 c.c. virus	Symptoms after 5 days; died after 17 days	Lesions of chronic cholera
	Weekly intervals	346	67	30 c.c. serum + 2 c.c. virus	Symptoms after 6 days; died after 21 days	Typical lesions
		347	60	2 c.c. virus	Symptoms after 7 days; died after 22 days	Typical lesions

Only the first seven bleedings were tested in this case. As the table indicates, the serum was not potent.

Hyperimmune 296.—Chester white, stag, weight 100 pounds. Injected May 29, with 40 Cc. serum (hog 221, 4th bleeding) and 2 Cc. cholera virus. One month later received 900 Cc. of 34-hour horse serum. Was not bled until August 1, practically two months after receiving the horse serum.

TABLE 13.

POTENCY TESTS OF SERUM FROM 296.

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
1 to 9..	1-32 days	343	64	40 c.c. serum + 2 c.c. virus	Remained well	
	Weekly intervals	344	77	30 c.c. serum + 2 c.c. virus	Remained well	
		347	60	2 c.c. virus	Symptoms after 7 days; died after 22 days	Typical lesions

The serum from the various lots of bleedings proved to be sufficiently potent to protect the test pigs against cholera.

TABLE 14.

TABULATED RESULTS OF EXPERIMENTAL HYPERIMMUNIZATION.

Hog	Results	Potency Established
173	Serum not potent	....
174	Serum potent	60 days
177	Serum potent	80 days
217	Serum potent	47 days
221	Serum potent	65 days
254	Serum potent	60 days
255	Serum potent	68 days
272*A*	Serum potent	80 days
288	Potency of serum below standard	34 days
291	First three bleedings not potent. Others not tested	Not determined
296	Serum potent	62 days

SUMMARY.

The results of these various tests relative to experimental hyperimmunization with horse serum virus suggest the following conclusions:

1. Cholera immune hogs can withstand intraperitoneal injections of large quantities of horse serum virus. Ten cubic centimeters per pound body weight can be injected without anaphylactic effects.

2. Horse serum virus when injected into immune hogs is capable of stimulating the formation of antibodies in the blood of the treated hogs.

3. Blood drawn from a hog at least one month after it has received an injection of horse serum virus is more potent than blood drawn at any earlier period.

4. Two injections (intraperitoneal) of horse serum virus, one month intervening, consisting of 10 Cc. and 5 Cc. per pound weight respectively, appear to produce a more potent blood than that produced by one injection.

5. It requires a larger dose of hyperimmune serum, prepared by this modified method, to protect against the test dose of virus, than is necessary with serum prepared after the original method.

II. FIELD TESTS WITH HYPERIMMUNE SERUM.

In order to determine the efficiency of the serum in immunizing against cholera under natural conditions, a number of field tests were conducted. The serum used in these tests had proven potent at the laboratory and included that from hogs 174, 177, 217, 221, 254, and 255.

Twenty-nine herds, located in three States, received treatment with the experimental serum. The animals in these several herds afforded conditions which are usually encountered in dealing with an epizoötic of hog cholera. The "serum alone" method of treatment was employed in every case.

The following is a description of each herd, together with the treatment and results:

Herd 1.—This herd, an infected one, was located near Flint, Mich., and at the time of treatment consisted of 270 hogs of various sizes and breeds. The disease had been present in the herd for two weeks, apparently having been introduced by a shipment of hogs received from the Detroit stockyards. Thirty hogs had died and 20 were sick, showing symptoms of cholera at the time of treatment (August 26). Three of the sick hogs were killed and postmortem examination revealed characteristic lesions of the disease. One hundred hogs were treated with the experimental serum (221). Of this number 22 were suckling pigs three weeks old; 52 weighed about 50 pounds each; 15 weighed 150 pounds; and 12 weighed 350 pounds each. The doses were 5, 25, 50, and 75 Cc. respectively. Thirty-three hogs were left untreated to serve as controls.

The final result of this experiment shows that 29 per cent of the treated hogs and 84.8 per cent of the controls died. Considering the fact that the herd was badly infected at the time of treatment the outcome of the experiment was quite satisfactory. No attempt was made to isolate the animals in any way during the experiment.

Herd 2.—This herd was located a few miles from Detroit, Mich. It consisted of 15 hogs at the time that the disease appeared. Two weeks later the number was reduced to seven. The disease was prevalent on adjoining farms. Six of the hogs weighing 50 pounds each were treated with 40 Cc. of serum from experimental hyperimmune 221. The remaining shoat was left untreated as a control.

The control and one of the treated hogs died about a week after the treatment. The other five treated hogs remained well.

Herd 3.—This herd was located in northeastern Kansas, in a vicinity where cholera had been prevalent for several months. It consisted originally of 38 hogs, but six of them had already contracted the disease and died. The remaining number had every chance for exposure, as they were in same pen with those which died. Fifteen 30-pound shoats and one 400-pound sow received treatment, consisting of 40 Cc. and 75 Cc. respectively. Three or four of the treated hogs showed slight symptoms of cholera when injected. Sixteen hogs were not treated. Serum 217 was used on this herd. The last report of the experiment shows that 93.7 per cent of the treated hogs and 100 per cent of the controls succumbed. In this experiment the serum had little effect in checking the disease.

Herd 4.—This herd was located one mile from herd 3. Cholera had been present in this herd for two weeks, during which time 40 hogs died of the disease. Of the 27 remaining hogs, 19 weighing approximately 70 pounds each were given injections of the serum (217), the dose administered being 40 Cc. One of these hogs as well as one of the eight controls showed evidence of infection on the day of injection. The final report of this test showed that 42.2 per cent of the treated hogs and 87.5 per cent of the controls died. The serum evidently gave some protection.

Herd 5.—This herd was located one-half mile from herd 4. It consisted of 61 pure-bred Poland Chinas of various ages. The herd had escaped cholera, which was present on adjoining farms. Thirty-five hogs, 16 weighing 75 pounds; three weighing 400 pounds; and 16 pigs three weeks old received treatment (serum 254), the remainder of the herd (26) serving as controls. Cholera entered the herd soon after the treatment, causing the death of 11.4 per cent of the treated hogs and 34.6 per cent of the controls.

Herd 6.—This herd was situated in the same neighborhood as the three preceding herds and though free from disease was in close proximity to it. Fifty-seven shoats were injected, each with 40 Cc. serum (255). Forty-one were left untreated as controls. Reports show that this herd escaped cholera, both treated and untreated hogs remaining in normal condition.

Herd 7.—Non-infected. Herd consisted of 42 shoats, average weight 70 pounds. Owner had visited infected herd on near-by farm and did not exercise any precaution against carrying the disease to his herd. Twenty-eight of the 42 hogs received each 40 Cc. of serum (255). Two weeks later one of the controls died, showing lesions of cholera. Three others were sick at the time, and were destroyed. The treated hogs were in a healthy condition, but owner sold them before completion of the experiment.

Herd 8.—This herd consisted of 76 hogs, mostly spring shoats, weighing 60 pounds, which were apparently in healthy condition. Disease present on farm two miles distant. Thirty-eight were treated with serum (225), receiving 40 Cc. each. The owner reports that the herd is still free from cholera.

Herd 9.—This herd consisted of 85 hogs. They had access to water that flowed through badly infected farms, but at the time of treatment they were free from cholera. Forty-two 50-pound shoats received 40 Cc. serum (217); 43 serving as controls. When the farm was visited one month later, all hogs were in good condition. It was reported later that this herd escaped the disease.

Herd 10.—This was an infected herd consisting of 125, 60-pound shoats, three of which were showing symptoms of cholera. Serum (254) was administered to 63. Two weeks later 15.8 per cent of the treated hogs and 19.3 per cent of the controls had died. Owner sold the remainder before definite results could be obtained.

Herd 11.—This was a non-infected herd located one-half mile from cholera. Of the 48 hogs in the herd 29 received each 40 Cc. serum (217 and 234). When hogs reached marketable age, they were sold, the entire herd having escaped cholera.

Herd 12.—This herd consisted of 32 head, 5 sows and 27 shoats. They seemed to be in good health, although across the road 40 hogs had died from cholera during the previous month. Three sows were treated each with 60 Cc. serum (217 and 255) and 20 shoats each with 35 Cc. of the same lot. Three weeks later 39.1 per cent of the treated hogs and 88.8 per cent of the untreated had died. The disease appeared in the herd a few days after treatment, six of the controls dying before any of the treated ones showed evidence of cholera.

Herd 13.—This herd located one mile from herd 12 was free from cholera at the time of treatment. Hogs on an adjoining farm were dying of the disease at the rate of 10 per day. Sixty hogs in herd 13 were injected with the experimental serum (255), leaving 77 as controls. Neither the treated hogs nor the controls became infected while they were under observation.

Herd 14.—This was a non-infected herd, consisting of twenty-two 60-pound shoats. There was no cholera within a radius of two miles. Sixteen of the 22 hogs received an injection of the serum (255). The entire herd remained well.

Herd 15.—This herd was located two miles from herd 14. Cholera had been present in this herd for three weeks, and of the original number of hogs (165) only 70 survived and practically all of these showed evidence of infection. Seventeen hogs, four sows, and 13 shoats received treatment, with a dosage of 60 Cc. for the former and 40 Cc. for the latter (serum 173). Twenty-eight animals which showed no symptoms were reserved as controls. One month later 5.9 per cent of the treated hogs and all of the controls had died. The serum proved of considerable value in this herd.

Herd 16.—In this herd 17 remained, out of a total number of 80. Infection had been on the premises for one month. Thirteen of the 17 were given 40 Cc. each of serum from experimental hyperimmune 174. The latest report from this herd shows that both treated and untreated hogs are well.

Herd 17.—This herd consisted of 30 hogs, three of which were sick at the time. The well hogs were placed in new pens and 19 of them (shoats weighing from 30 to 75 pounds) were injected with serum (217). Five weeks later 47.3 per cent of the treated hogs were dead and 45.4 per cent of the controls. Most of these hogs died during the week following the injection, indicating that they were developing the disease when treated.

Herd 18.—This herd was located four miles from cholera, but the owner intended to transfer the hogs to an infected farm four weeks later. Twenty-four were treated with serum (217), 45 Cc. being administered to each, and six were kept as controls. A report from this herd six weeks later shows that all of the controls and one of the treated hogs succumbed to cholera.

Herd 19.—This was a healthy herd of 65 hogs. Proximity of disease, one mile. Fifty-one were treated with serum (173 and 177). Of this number 48 were 60-pound shoats and three were sows weighing about 300 pounds each. Serum injected in amounts of 40 Cc. and 75 Cc. Both treated and untreated hogs remained well.

Herd 20.—These hogs were the property of a cattle feeder and had been purchased from neighborhoods where cholera was prevalent. At the time of treatment they appeared to be healthy. Twenty-nine of the 38 hogs received treatment, which consisted of an injection of 40 Cc. of serum (177 and 217). The final results of this experiment show that 88 per cent of the controls and 51 per cent of the treated hogs had died.

Herd 21.—This herd consisted of 16 shoats, averaging in weight 50 pounds. Twelve of these were treated each with 40 Cc. serum (173). These hogs as well as the controls withstood an infection which was present on adjoining farm.

Herd 22.—In this herd of eight hogs only three were treated, two of which had a temperature of 104.5° at the time of treatment. Both of these hogs died. The other treated hog and two controls survived. Serum used, 254. Dosage 40 Cc.

Herd 23.—Infection not present on farm. Treated 24 hogs, leaving three as controls. Serum 254 was used. This entire herd remained well as long as observation continued.

Herd 24.—Five hogs were treated with serum 253 and 255 in doses of 40 Cc. Two controls. Latest report indicates that cholera had not appeared in the herd.

Herd 25.—In this healthy herd of 14 head, 13 received treatment with serum 255. The disease was present on neighboring farm. A report from this farm six weeks later showed that all hogs were healthy.

Herd 26.—This herd consisted of three 50-pound shoats. Two were injected with serum (254) in a dose of 40 Cc. each. Final result, control dead and both treated hogs well.

Herd 27.—Fifteen hogs in herd. Two died a few days previous to the beginning of the experiment. Ten were injected with 35 Cc. of serum

(254). Five weeks later the disease had run its course, with the result that two of the control hogs and none of the treated died.

Herd 28.—One hog treated, three controls. Treated hog received 60 Cc. of serum (254). The result was that the treated hog remained well and two of the controls died.

Herd 29.—There was an infection one-half mile from this herd. Ten of the hogs were given the serum treatment, a dose of 70 Cc. being administered (221). The latest report shows that cholera has not entered the herd.

TABLE 15.

SUMMARIZED RESULTS OF FIELD WORK.

Condition of Herds	Number of Hogs Treated	Number Died	Percentage	Number Controls	Number Controls Died	Percentage
Not exposed before or after treatment.......	371	0	0	267	0	0
Exposed before treatment and infection evident..	308	99	32.1	191	122	63.8
Exposed, no symptoms before treatment... ..	87	5	5.7	46	19	41.3
Total..	766	104	.	504	141	....

A general survey of the field tests, as well as of those conducted at the laboratory, indicates that the experimental serum used in this work possessed protective properties. Of the 29 herds treated, 13 were infected at the beginning of treatment. The disease was of an extremely virulent type in four of these herds, namely, numbers 1, 3, 4, and 12. In the infected herds a total of 308 hogs received treatment and 191 served as controls. The final results show that 67.9 per cent of the treated hogs and 36.2 per cent of the controls survived, which points to the fact that the serum had both a prophylactic and curative effect. In the case of the non-infected herds only three of the 16 became exposed after treatment. In the three herds which became exposed to hog cholera after experimental treatment (herds 5, 7, and 18) the average final result is expressed by a survival of 94.3 per cent of treated hogs and of 58.7 per cent of the controls.

GENERAL CONCLUSIONS.

The practical use of horse serum virus in the preparation of hyperimmune serum is open to question. The method, in so far as our results show, has two disadvantages:

1. The animals used for hyperimmunization must be kept under treatment several weeks longer than when treated by the original method.

2. A larger dose of the serum, as compared with that which is recommended by those engaged in preparing hyperimmune serum according to the original formula, must be administered.

This work has resulted in further experimental evidence that horse serum virus represents an activated hog cholera virus. It would scarcely appear possible to produce hyperimmune serum as relatively potent as that which has been used in this work, were similar dilutions of the original cholera virus, in physiologic salt solution *in vitro*, used for the purpose of hyperimmunization in substitution for "horse serum virus."

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STUDIES ON THE VIRUS OF HOG CHOLERA.*

PRELIMINARY REPORT.

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This preliminary report is presented for the purpose of recording certain observations, which have been made by the aid of the dark field on the blood of hogs suffering from hog cholera. During the last few months a spirochete has been found with uniformity and constancy in the blood of every cholera hog examined. Practically all of these positive findings have been controlled by one or more careful dark field examinations of the blood before inoculation. Additional checks are furnished in several cases by negative findings subsequent to positive results in blood of hogs recovered from the disease.

In this work the fresh blood of normal and diseased hogs is collected aseptically in sterile sodium citrate solution. It is then examined at once with the dark field. The blood of normal hogs thus examined may show, in addition to the normal structures, a few granular bodies, sometimes a few bacterial cells from possible extraneous contaminations, and filaments. The latter may assume the form of "dumb bells," "chains," or flexible filaments, which by an untrained observer might easily be mistaken for spirochetes. An excellent description of these bodies as well as others found in the blood of normal animals is given by Balfour.[1]

In so far as the present results go, the practiced observer can readily distinguish certain characters in the blood of animals suffering from hog cholera when placed on the dark field, as differentiated from normal hog blood. It usually contains many granules, some very fine yet distinctly larger than blood dust, some larger still, and some very distinct, highly refractive bodies. It is possible that some or all of these granules represent disintegrated blood elements resulting from the disease.

In the specimens of blood from all infected hogs which have been observed on the dark field, a relatively large spirochete has

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[1] *Fourth Report, Wellcome Tropical Research Laboratories.*

been found. It averages from five to seven microns in length and one micron in width. The body of the organism is flexible, round and blunt at its ends. It is actively motile and revolves about its longitudinal axis. Its motility is undulating in character and its spirals are fixed. Several of these have been observed dividing longitudinally. In one permanent microscopical mount, prepared by india-ink fixation, one of these organisms shows a polar flagellum. This spirochete is readily distinguished from bacteria on account of its lack of rigidity and characteristic motility, and from "blood filaments" by its refractive properties and characteristic morphology.

To date, positve findings of this spirochete are recorded in the following strains of virus:

1. Michigan strain of hog cholera virus (originally B.A.I. strain of virus).—In the blood of seven hogs, all checked by dark field examinations of normal blood, except the first one, in which was observed the spirochete.

2. Michigan strain (Demerick farm). In six hogs all checked by normal examinations, except the first two, which were suffering from hog cholera when received from the owner.

3. Indiana strain.—In one hog controlled by negative findings in blood 24 hours after inoculation.

4. Michigan strain (Rochester).—In seven hogs all of which were showing symptoms when received. In these animals diagnosis was practically made by means of the dark field.

5. California strain.—In one hog, checked by repeated examinations of blood before inoculation.

6. Kansas strain.—In one hog checked by examination of blood before inoculation.

7. Ohio strain (Pettigrew).—In one hog checked by examination of blood before inoculation.

8. Unknown (mixed) strain of virus.—In four hogs, infected through natural exposure. These hogs were supposed to be healthy, and after obtaining positive findings in the blood of one, physical inspection showed that all were manifesting early clinical symptoms of hog cholera.

9. Controls are furnished by repeated negative findings in two untreated animals kept for a time as checks, by repeated negative findings in four "natural immunes," and in six animals which became convalescent and finally recovered.

To date 33 hogs have been under observation, 130 dark field examinations have been made, and permanent microscopical preparations of the organism in the B.A.I., Indiana, and Rochester strains of virus have been secured.

Two experiments have been made relative to the horse serum virus phenomenon,[1] which showed the presence of the spirochete in the horse serum virus.

The data thus far collected in this investigation are limited and do not warrant the formation of definite conclusions as to the etiological significance of the findings. The details of the experiments and additional material will be presented in a later publication.

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[1] King and Wilson, *Jour. Infec. Dis.*, 1912, 11, p. 441.

6-855

THE PHYSIOLOGICAL ACTIVITY OF CANNABIS SATIVA.

COMPARISON OF EXTRACTS FROM INDIAN AND AMERICAN-GROWN DRUG UPON HUMAN SUBJECTS.

H. C. HAMILTON, A. W. LESCOHIER, R. A. PERKINS.

It has been claimed by various investigators that the common hemp (*Cannabis Sativa*) grown in the United States contains the same active constituent as is found in Cannabis Indica, the name of the official drug which is grown in India. Botanists do not distinguish between the two, the plant being identical wherever grown.

The fact that the Indian-grown drug was used in all the early accounts of its intoxicating action may have led to the belief that the peculiar climate of India is accountable for the presence of an active constituent not normally present in the plant.

No recorded data have been advanced, however, to substantiate the claim that drug grown elsewhere does not contain such constituents. On the other hand, Wood (Proc. Am. Phil. Soc., Vol. XI, p. 226), Houghton and Hamilton (*Am. Journal of Pharmacy*, January, 1908), True and Klug (Proc. A. Ph. A., 1909), True (*Am. Journal of Pharmacy*, January, 1912), and Hamilton (*Am. Journal of Pharmacy*, March, 1912), have submitted the drug to careful pharmacological tests, and report that extracts from American-grown drug are no less active than those obtained from India.

Dr. H. H. Rusby raised the question whether the test for activity on dogs can be accepted to prove its activity as a therapeutic agent.

Much of our knowledge of the action of drugs is obtained by observing their effects when administered to animals. The physiological action of almost every powerful drug is so characteristic as to be almost unmistakable to an experienced observer. Any one who has observed the characteristic effect of Cannabis Indica on susceptible dogs, symptoms which almost invariably appear in an hour after administering one to two grains of an active extract, and then has observed the same effect from an equal dose of an extract from the American drug, is inclined to accept it as proved that the two are identical.

The question raised by Rusby is, however, very pertinent and logically calls for proof of a different character. A series of experiments was therefore outlined which, it was hoped, would throw light on this much mooted question. To make a complete experiment it was decided that three persons would coöperate, each in turn, taking the same quantity of each lot of drug, while two would remain normal to observe its effect.

There is not much of interest in observing the effect of the drug on others, since its action is more mental than physical. One's own description, if it could be recorded at the time, would mean much more than that of others. The subject, however, is not in a condition at the time to record these observations, and if of a nervous disposition needs the presence of companions. Otherwise drowsiness is often the most characteristic effect of the drug.

The evening was taken for these experiments, partly to give opportunity for sleep immediately afterwards and partly to have everything quiet with no disturbing affairs going on to distract attention.

One of the three (Hamilton) had on a previous occasion taken two grains of an active extract Cannabis Indica and was, to that extent, familiar with its action. On that occasion there were developed some disagreeable symptoms but nothing serious.

Nausea and vomiting occurred, which were magnified by the imagination to an extent that was far from pleasant. Therefore, to duplicate conditions as nearly as possible the capsule containing 2 grains extract Cannabis Americana was taken at 5:30, followed by dinner at 6 o'clock.

EXPERIMENT I.

H. relates his experience as follows:

About one hour after taking the drug a pleasurable sensation was experienced which can be described only as one of well-being and complete satisfaction. This was marred to an extent by the dread that the trip to the laboratory might not be entirely comfortable, and that in the street-car or on the street my behavior might be ridiculous without the cause being known. The walk to the car, the two-mile ride, and several blocks walk to the laboratory, seemed interminable, although no unpleasant feelings were experienced during the trip. One other fact was observed, namely, the difficulty in holding my mind on one subject long enough to express my thoughts.

About two hours after taking the drug, an uncomfortable feeling was experienced, followed shortly by nausea and vomiting. Several ideas impressed me strongly; I had a morbid fear that some one other than my associates would observe me, also that the effect of the drug on me would deter the others from taking it. I was opposed to doing anything and wished most earnestly for a comfortable seat or bed. A feeling of constriction and dryness in my mouth and throat was observed. Later a feeling of depression and drowsiness followed and I appeared to sleep. Whether I did or not is uncertain as I thought I remained conscious all the time. I knew that something in my condition was decidedly abnormal because of comments made by the observers, but I didn't know nor care what it was.

About four hours after taking the drug I felt much better and aroused entirely from my drowsy state. On the trip home I dozed off on several occasions, but for only a few minutes each time. A comfortable night's sleep followed and no unpleasant after effects could be noticed.

The result of this experience convinced me that no difference could be detected in the action of extracts from Indian and American hemp, for, although in the former experiment there were several phases which did not appear in this one, the general effect was identical in each case. On the former occasion all the peculiar sensations were more vivid, time dragged more slowly, the nausea was greater, even suggesting the fear of death, the constriction in the throat was so great as to suggest choking to death, there was a greater willingness to give free rein to my imagination and to relate experiences, and therefore greater difficulty in keeping the mind on one subject at a time. These differences were, however, in degree and not in kind and may be explained in part by my having become familiar with the drug and descriptions of its effect on others.

L.'s Observations on Subject H. Ex. I. About 6:30 H. began to manifest a certain amount of uneasiness and difficulty in concentrating his thoughts. Coming from down town to the laboratory it was observed that he seemed to be more or less worried and to lose, to a certain extent, sense of time, expressing the feeling that we had consumed an hour coming from down town, whereas the time for the trip was not more than ten or twelve minutes.

The laboratory was reached at 7:00 and H. expressed a strong desire to lie down or become ensconced in a comfortable chair. From 7:00 to 7:30 he appeared generally depressed and became irritated about seemingly trifling matters. At 7:40 pulse was taken and found to be 120, weak, irregular and easily compressible. Skin was cold and clammy and he expressed a belief that he was going to be nauseated. 7:50, pulse had dropped to 96, but was still weak and irregular.

8:00	Pulse	92.	Severe vomiting.
8:15	“	96.	Vomited.
8:30	“	88	
9:00	“	84	
9:30	“	86	
10:00	“	96	

The last record was taken after H. had been up walking around the room, which undoubtedly accounts for its increase over the one previously taken. It was observed throughout that when H. exercised, even to a slight extent, the heart action was markedly accelerated. In one instance the pulse rate was taken immediately after H. had been walking and was found to be 96. When taken less than a minute afterwards it was about 80, and was again increased to 96 by comparatively slight muscular movement. The pulse rate varied from 96 to 80 or 82 within a minute's time. Throughout it was soft and obliterated by slight pressure. During the whole evening his ideas seemed to be more or less confused, and it was apparently impossible for him to concentrate his thoughts on any particular subject. After beginning to make a remark, he would lose entirely his trend of thought, and be quite unable to complete it. At 10 p. m. the more marked effects of the drug had worn off.

P.'s Observations on Subject H. Exp. I. H. showed no symptoms whatever until about 6:30, when it became evident that he was worried and somewhat nervous. He said that the effect of the drug was coming on and expressed a desire to go to the laboratory as soon as possible. On the way he worried and fretted, at times fearing that he would be unable to walk and would make a spectacle of himself before reaching the laboratory. However, nothing of particular interest happened during the trip except an evident lapse of memory and evidence of nervousness. On arriving at the laboratory he expressed a desire for a comfortable chair or a bed and complained of feeling sick at his stomach. He was pale and his skin was cold and moist. Before long he vomited freely. This was repeated after a few minutes, but did not seem to relieve him greatly. He complained of a dryness in his throat and was continually wetting his lips. His pulse rate was almost alarming, varying greatly in rate from 84 to 120 within a minute, but for the most part being very fast and weak. His skin was cold and clammy and respiration somewhat shallow.

For over two hours he lay back in his chair in a sort of stupor, seeming to be asleep, but easily aroused. He had no disposition to attempt anything, not even to talk. During the early part of the evening he was evidently much worried, fearing that his condition would deter his colleagues from taking the drug. He also seemed to have a dread that some one other than those associated with him in the experiment would see him. He was asked to write, but firmly refused even to attempt it. When asked if he were having beautiful dreams and visions, his only reply was, "I wish I could tell you." He remained in this semi-conscious condition until about 10 o'clock, when suddenly he aroused himself, said he felt all right and was ready to go home.

He dozed off momentarily twice on the car, and felt all right the next day except for a very faint headache.

EXPERIMENT II.

L.'s Personal Experience. A 2-grain dose of solid extract Cannabis Americana was taken upon an empty stomach. For two hours no sypmptoms of any kind were experienced. Then there was a peculiar unnatural sensation. This initial manifestation is difficult, in fact, impossible of description. No distress was evidenced nor was the feeling exceptionally pleasant. It was simply a recognition of the fact that I was not quite myself. Following this period there shortly developed a feeling of great elation, and a sense of well being. With no particular reason for being so, I felt inexpressibly happy. There was a twitching and drawing of the corners of my mouth and an uncontrollable desire to laugh, although I could not laugh aloud. Everything pleased me and I felt that my happiness was absolutely complete. The only tinge of regret that I experienced was that my colleagues were not having the same delightful experience. The more marked effects of the drug appeared to come in waves, although the general sense of elation was never lost. An occasional undulation would sweep over me and I would feel as though my body was swaying, and there was an inclination to strike the table with my hands in an exuberance of delight. At times I had great trouble in coördinating my thoughts, although between the paroxysms which have been described, my mind seemed reasonably clear. I felt that I was acting in an exceedingly foolish manner, but had no power to control myself and in fact did not care to. As it grew late in the evening the stimulating effects of the drug decreased and I became somewhat irritable and touchy about trifling matters. At 10 o'clock the greater part of the effects had worn off, although I did not feel entirely normal. After a light lunch I retired and slept very soundly. No after effects of any kind were experienced on the following day.

H.'s Observations on Exp. II. L.'s experience was almost entirely one of enjoyment. There was no nausea and no evident discomfort, although he once remarked that the earlier effects were much the more pleasant. There was unquestionably the same well-being, expressed by his repeatedly saying, "I feel so good." Hearty laughter for which there was no evident reason was explained in this way. At no time was there any desire to carry on conversation more than to answer any questions addressed to him. This would account for there being no noticeable difficulty in keeping his thoughts collected.

Later a sensation of drowsiness was evident and with it expressions of irritation when anything of a disturbing nature was said or done. The effect of the drug was long delayed in appearing, nothing being noticed either by himself or the others until nearly two hours after its administration. This probably explains why its effect was so persistent, intoxication being very evident fully six hours after the drug was taken.

P.'s Observations on Exp. II. No effect was noticed for about two hours, when a slight twitching of the corners of the mouth was observed and a tendency to smile. When asked why he smiled he said he didn't know, just felt good but could not define the sensation, it was simply one of enjoyment. He said that he felt sorry for us, as he was the only one enjoying himself. Presently he broke into a restrained but hearty laugh. When questioned, he said it was simply because he couldn't help laughing. He admitted that he was making a fool of himself, but said he couldn't help it and didn't care anyway. At one time he pointed at an article of furniture in the room and had another laughing spell. When asked the reason he merely said that it was funny. He answered all questions put to him, but showed no tendency to be talkative, most of his answers being short.

These spells would last for probably a minute or two and then there would intervene a normal period of ten to twenty minutes. He said he was simply "happy" drunk, and he looked and acted that way. Later in the evening he showed a decided disposition to be annoyed by talking or answering questions and remarked that the earlier effects of the drug were much the more pleasant. At 10 o'clock the action of the drug had worn off sufficiently so that he felt inclined to go home. He was somewhat irritable on the walk from the laboratory and said afterwards that he was very drunk on the way home. He ate lunch before retiring and enjoyed a comfortable night's sleep and felt fine the next day, with no bad effects whatever. Observations were taken of the blood pressure (systolic) and of the pulse rate at intervals during the evening, but nothing abnormal was noticed. The pulse was full and steady and the rate averaged about 80, not varying more than six beats at any time. The blood pressure was 130 mm. of mercury throughout the evening.

EXPERIMENT III.

P. relates his own experience as follows: At 4:30 I took a capsule containing two grains S. E. Cannabis Americana on an empty stomach. About one hour later, while talking to my colleagues about the best time for them to go out for a lunch, they asked me if I didn't feel anything; I answered, "No," and truthfully I did not, but no sooner had I spoken than I experienced a peculiar sensation. The corners of my mouth commenced to draw and I could not refrain from laughing; I laughed so heartily that I was tired afterwards, although nothing seemed particularly amusing. This spell lasted for probably half a minute, although it seemed much longer to me.

Then my associates left me, and I was alone in the laboratory. At this time I felt most exhilarated. Everything seemed so enjoyable and I was extremely comfortable. I walked up and down the corridor, swinging lightly along, seeming to walk on air or feathers. My feet weighed nothing. It was no effort to walk; it was more like floating along. My sense of proportion was lost, my feet seemed miles away from me, my arms were long and big. The corridor was miles long; I walked or rather floated up and down apparently for hours, waving my hands and arms, marking time to imaginary music. All this while I was smiling and enjoying myself immensely. All my faculties were not impaired, however, because to test myself I read part of a typewritten notice on the bulletin board. I was standing there when a person who knew nothing of the experiment passed by. We greeted each other, and evidently he noticed nothing peculiar in my appearance nor actions. I was surprised at this, for it seemed to me that he must see how silly I looked and how I swayed when I walked, but especially he should have noticed my voice, which sounded to me like the deepest bass. It seemed to me to be musical and full toned and I liked to hear myself talk. My colleagues, however, did not seem to notice it, nor did they appreciate that I felt so good toward them and myself.

After what seemed hours of walking I sat down to await their return from lunch. Several waves swept over me during this time and also later on, which are very difficult to describe adequately. The feeling was one of well being and perfect satisfaction, beginning with a sort of numbness or fulness in the extremities, a feeling of unreality in the surroundings. I knew that my hands were normal in appearance, but when not observing them, they seemed to be detached and not a part of me. We played a game of cards, and in playing a card I seemed to be throwing some enormous but very light article over a great distance. These spells usually started by smiling and ended in laughing rather hysterically, pounding the table with my fist. But I could not laugh aloud because of the peculiar drawing and constriction about my face and neck previously noted. As the effect began to wear off these paroxysms became less frequent but no less irresistible. I felt no unpleasant symptoms at any time. About 10 o'clock I was hungry and ate some sandwiches with great relish before going home. I reached home without any difficulty, not feeling drowsy and without any change in my feeling of enjoyment. Upon arriving home I retired immediately because I felt that I was not entirely normal. Before going to sleep, however, I experienced another wave.

I awoke early the next morning very much refreshed and none the worse for my experience.

H.'s Observations on Exp. III. The experience of P. was practically a duplicate of L.'s. The effect appeared one hour after taking the drug, and except for an occasional lapse his normal condition was regained five hours afterwards. There was more uncontrollable laughter in his case, no irritability and no apparent discomfort at any time. He seemed to give himself up more completely to the enjoyment of his sensations than the others. At times he seemed to be addressing an imaginary audience, pacing back and forth, gesturing and appearing to talk to himself.

We were inclined to question whether some of his actions were not assumed and voluntary; but he assured us that he was acting just as he felt.

L.'s Observations on Exp. III. P. began to feel the effect about an hour after the administration of the drug. He seemed to be possessed of a desire to move about, paced up and down the corridors, declaring he felt as though he weighed not more than fifteen pounds. He was apparently very much pleased with himself, and bubbling over with happiness. At times he would be seized by fits of uncontrollable laughter, which in some cases was spontaneous and without apparent cause, but usually it was incited by the others laughing at or with him. Between these paroxysms of laughter P.'s condition was practically normal, he could talk rationally, and his mind, as far as indications could be depended upon, was clear. At no time did there seem to be a loss of coördination. It was observed that the action of the drug was apparently produced in waves, while between these seizures one's condition would be practically normal.

During the three experiments recorded above, the one under observation felt a certain restraint, knowing that the others were watching for every abnormal action. For this reason it was decided to vary the conditions in the further experiment and have all three under the influence of the drug at the same time. It was hoped in this way to eliminate the restraint evident in each of the individual experiments and perhaps observe some new features in the action of the drug.

EXPERIMENT IV.

In this experiment H. took Extract of Cannabis Americana again, while L. and P. took extract Cannabis Indica. This gave an opportunity for L. and P. to compare the effect of the two varieties, both on themselves and on the others, while H. took this opportunity to repeat the experiment with all the conditions the same, except that he ate no dinner until the effect was practically gone. All three took the drug at 4:30 on empty stomachs, the dose in each case being two grains.

This last experiment, while not developing any new features, was in other respects successful. H. had no unpleasant experience and the evening was one of unalloyed pleasure, proving that all the discomfort was directly traceable to the nausea from having food in the stomach. L. considered the effect to be much less intense and of shorter duration in this experiment than that from the American drug, while P. took the opposite view in his case.

H.'s account of the experiment is as follows: L. was the first to note the characteristic effect of the drug, while P. and I remained unaffected for fully two hours after it was taken.

The same feeling of well-being and complete satisfaction was experienced by all, this being as evident to the observers as to the subject himself. Uncontrollable laughter was more frequent and longer continued than in the individual cases, probaby because during a cannabis intoxication so little is necessary to excite it, and when one started the others joined in the hilarity. No one felt inclined toward any activity, but only to give himself complete relaxation. Each of the three was emphatic in stating that he knew when he was making himself more or less ridiculous, but could not control the impulse nor did he wish to restrain himself.

About six hours after taking the drug, at the end of a quiet card game, without any comment, each of the three assumed as comfortable a position as possible and fell into a doze. It was apparently not sleep in any case, as each was fully conscious of noises in the building and annoyed by them.

This lasted not more than ten minutes, at the end of which we all felt fully aroused and ready for something to eat. This ended the experiment as outlined in advance. The only variation from the original plan was, as noted, for all three to experience the effects at the same time. No point was lost because of this, since the subject is at all times acutely conscious of everything occurring.

L.'s Account of Experiment IV. My personal experience with Indian Cannabis was very much the same as those already narrated as occurring with the Cannabis Americana, although the effects were developed somewhat more promptly, and were not quite so pronounced or lasting. P.'s feeling seemed also to duplicate very closely those which he had had from the Cannabis Americana, but contrary to my own were somewhat more pronounced. H. did not have any of the nausea or any of the other uncomfortable features which occurred during the first experiment, indicating very clearly that these symptoms were due to the hearty dinner which he had eaten, and were not to be construed as characteristic of Cannabis. The drug in this last experiment was taken at half-past four, and the greater part of the effects were felt from about half past six to eight o'clock. After that time the more exhilarating action had worn off, and I experienced only a drowsiness. For a half or three-quarters of an hour after I had ceased to feel any more marked effects of the drug H. and P. continued to be very much exhilarated. About 9 o'clock all three of us became drowsy, and as if by mutual consent laid our heads on the table in a sort of doze, although none of us really went to sleep. This condition continued about ten to fifteen minutes, after which we felt much refreshed.

P.'s Account of Experiment IV. L. was the first one to show any symptoms from the effect of the drug. He had practically the same experience as on the previous occasion. H. and I did not feel any effect for fully an hour later than L., but finally went under the full influence of the drug very suddenly, there being no premonitory symptoms whatever. At times one of the three would have a paroxysm of laughter alone, but usually one would start laughing and the others join him at once. It was observed, however, that L. was getting over his intoxication early, and he sat there seemingly rather bored and provoked at the others for being so happy. The effect on myself was apparently more intense than that of the previous test, and more so than was experienced by the others, laughing spells being more frequent and inclined to be hysterical. No unpleasant symptoms were experienced by any one of the three during the evening. After several hours playing cards and talking a peculiar thing happened. Suddenly and without a word from any one we stopped the game, lay back in our chairs and dozed. It seems to me that I slept for a long time, although it was in reality only about ten minutes. It probably was not really sleep as I remember hearing the watchman on his rounds, and I wondering whether he would come into the room where we were. As suddenly and spontaneously as we had dozed, we aroused and, having practically recovered from the effects of the drug, prepared to go home.

CONCLUSIONS.

It may be stated with certainty that the physical and mental condition of the human subject at the time of administering this drug influences its effects both in degree and kind. For that reason no two persons can be expected to exhibit the same symptoms as a result of ingesting equal quantities of the same drug, and no person can be depended upon to react in exactly the same manner from the same drug on different occasions. With these facts in mind the differences in the three personal experiences above related are readily explainable, and there is no reasonable ground for doubting that Cannabis Sativa grown in India and America contains the same active constituent.

The method of assaying extracts of Cannabis Sativa described in detail by Houghton and Hamilton (*Am. Journ. of Pharm.*, January, 1908) makes use of dogs for exhibiting the characteristic effect of the drug. Attention is called in this article to the fact that the animals must have been specially selected for the purpose. They must not only be susceptible to the drug but their behavior under its influence must have been determined by preliminary observation. We may thus avoid errors due to their individual idiosyncrasies. There are, apparently, no such marked differences in the character of the reaction in dogs as are observed in human subjects, nor are they so variable at different times if they have been carefully selected as described above.

When proper precautions are observed the activity of an extract Cannabis Sativa relative to a standard extract may be determined with reasonable accuracy. Twelve years' experience in observing tests of Cannabis Sativa obtained from different countries, Africa, India, Germany, Greece and various localities in North America, has supplied data to prove that they all contain the same active constituent.

THE IODINE CONTENT OF THE SMALL, MEDIUM AND LARGE THYROID GLANDS OF SHEEP,* BEEF AND HOGS.

By T. B. Aldrich, Ph.D.

(From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.)

It is conceded by a majority if not all writers on the subject of thyroid therapy that the thyroid gland (or its preparations) to be physiologically active must contain at least *some* iodine; furthermore, that this iodine to be of the greatest value therapeutically must be combined or associated with some protein or organic complex found in the gland. Presumably the iodine is the more important constituent; the two, however, associated or combined seem to give the best therapeutic results, and to-day the efficiency of a thyroid preparation is generally measured, or should be, by its iodine content. In fact, for some time a number of pharmaceutical houses have been putting out thyroid preparations with a guaranteed percentage of iodine. Since then the iodine content of a thyroid preparation is a measure of its therapeutic efficiency, it is desirable to select, if possible, those glands which contain the most iodine, providing other factors are equal, and from those animals whose thyroids contain the most of this constituent.

The following work was taken up with the object of determining the iodine content and thereby the therapeutic efficacy of some thyroid glands, especially the small, medium and large thyroid glands of sheep, beef and hogs, by means of the method employed by Hunter (*Journal of Biolog. Chemistry*, 1909-1910, VII, p. 321), which is very accurate and detects the presence of very small amounts of iodine that are incapable of being detected by the older method of Baumann (*J. Physiol. Chem.*, 1896, 21, p. 489; *Ibid.*, 22, p. 1), which has been the method usually employed heretofore. (See Rigg's work, *J. of American Chem. Soc.*, 1910, XXXII, p. 692; *Ibid.*, 1909, XXXI, p. 710.)

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*The iodine content of some mixed sheep thyroids was also determined.

The glands received came from the Chicago stock yards, were placed in Mason jars as soon as removed from the animals, subsequently placed in cold storage and shipped packed in ice. They were all received in the best possible condition. Six lots were obtained, as follows:

(1) Mixed sheep thyroids (Lots A and B).
(2) Small, medium and large sheep thyroids (Lot C).
(3) " " " " " " (Lot D).
(4) " " " " beef " (Lot E).
(5) " " " " hog " (Lot F).

In Lots A, B and C the glands were not counted.

After freeing from superfluous tissue and weighing, the glands were ground very fine, defatted and desiccated in the usual manner, and eventually reduced to a very fine powder by passing through a 60-mesh sieve.

The following information obtained by one* of our staff from a packer, relative to thyroids, may be of interest at this point.

(1) Sheep thyroids are subject to great variation in size. The sex factor is not the determining factor for the size of the gland, nor has the condition of nutrition of the sheep any decisive bearing on the size of the gland. They run from the size of an almond to the size of a lemon.

(2) Steers' thyroids are larger than cows', this variation in size being a *constant* factor; size of gland varies again with the condition of the animal. Well-nourished cattle have larger thyroids than poorly-fed ones.

(3) In hogs, the thyroids vary little in size and present only slight variation in general appearance.

The thyroids of cattle are removed after head has been severed; same is true of hogs. Cattle thyroids are often cut, those of hogs not.

The following method of assaying the iodine, somewhat abbreviated, was employed. (For details see Hunter, *The Journal of Biological Chemistry*, 1909-1910, VII, p. 321.)

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*It is a pleasure to thank Dr. Baeslack, of our staff, for looking after the collection of the glands and also for the information relative to the same.

Exactly one gram of the body was taken, placed in a nickel crucible (125 c.c.), 14 grm. of the following oxidation mixture added (106 parts Sodium carbonate, 75 parts Potassium nitrate, and 138 parts Potassium carbonate), and the two intimately mixed by means of a nickel spatula. Over this was dusted 4 gm. of the oxidation mixture. The nickel crucible was then heated over a flame until contents of the same was perfectly white. This more or less fused mixture was dissolved in water and brought into an Erlenmeyer flask (800 c.c.). After cooling, 35 c.c. of Sodium hypochlorite solution was added, and while holding the flask in a slanting position in cold water and agitating at the same time, 65 c.c. of 42½% phosphoric acid was added. The solution should, after the addition of the acid, be colored slightly yellow from the slight excess of chlorine liberated. The mixture was then boiled briskly, a funnel with a short stem being placed in neck of flask to avoid any loss. When all the free chlorine was expelled, recognized by holding filter paper moistened with starch solution containing Potassium iodide in the steam (blue color if present), the flask containing now about 70-80 c.c. was allowed to cool and brought up to about 200 c.c. by the addition of water. To this cold solution 10 c.c. of a 1% Potassium iodide solution was added, which causes the liberation of six times the amount of iodine originally in the product to be assayed, according to the following equation:

$$KIO_3 \cdot HIO_3 + 10\ KI + 11\ HCl = 11\ KCl + 6\ H_2O + 12\ I$$

This liberated iodine is immediately titrated with a standard solution of Sodium thiosulphate solution approximately N/200, a few drops of starch solution being added toward the end of the reaction.

The number of Cc. of Sodium thiosulphate used multiplied by the iodine factor then divided by 6 gives the amount of iodine in the original sample.

A blank test using casein, or some other body free from iodine, was made to insure the absence of iodine in the reagents, and the usual precautions employed in analytical methods observed.

The following table gives the number (except in the lots A, B and C), total weight of glands, average weight of glands and iodine content in each lot with average iodine content and percentage in each gland, where the number of glands is known.

SHEEP THYROIDS (LOTS A AND B)

	No. of Gland	Total Wt. of glands gms.	Av. Wt. of glands gms.	Iodine % (moist gland tissue)		Av. iodine in each moist gland mg.	%
(A)		2925 (MX)*		.032	.025		
(B)		5120		.022			

SHEEP THYROIDS (LOT C)

(1)		6000 (S)		.027	.019		
(2)		5200 (M)		.018			
(3)		5805 (L)		.01			

SHEEP THYROIDS (LOT D)

(1)	397 (S)	540	1.36	.014	0.028	.6	.04
(2)	192 (M)	650	3.38	.028		.9	.027
(3)	48 (L)	675	14.00	.015		2.1	.015

BEEF THYROIDS (LOT E)

(1)	98 (S)	620	6.32	.039	0.036	2.47	.04
(2)	53 (M)	405	7.64	.030		2.40	.031
(3)	34 (L)	425	12.20	.038		4.70	.038

HOG THYROIDS (LOT F)

(1)	108 (S)	725	6.7	.054	0.047	3.6	.05
(2)	70 (M)	765	10.9	.048		5.2	.047
(3)	40 (L)	735	18.37	.041		7.5	.04

The average iodine content of the mixed sheep thyroid glands, Lots (A), (B), (C), and (D) where over 50 lbs. were employed, is about .025%, while in some cases with selected glands 0.014% has been obtained and .027 where relatively large amounts (6000 gms.) were used.

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*The letters (S), (M), (L), and (MX) stand for small, medium, large and mixed glands.

The beef thyroids (Lot E) gave an average of .036% iodine, and in selected cases nearly .04%. The hog .047% average, in selected glands over .05% iodine.

It will be noted that the greatest variation in iodine content in the different sized glands of the same animals is in that of the sheep, where it varies from .01%-.027% in Lot C and from .015-.044 in Lot D. This variation being no doubt due to the greater prevalence of goiter in sheep.

Next to sheep the iodine content of the hogs' thyroids vary the most, .041-.054%, while in the beef we have the least variation, .030-.39%.

The hogs' thyroids* contain the highest percentage of iodine, with the small sheep glands in one case higher, in the other lower than the mixed beef glands. Assuming the iodine content to be a measure of the therapeutic activity, the mixed thyroids of the hog are superior to the beef and the latter superior to sheep thyroids, small sheep thyroids being about equal to mixed beef thyroids.

Weight for weight the small glands of all the animals studied, nearly without exception contain the most iodine (excepting beef, where the large and the small have nearly the same, .038%).

In general the larger glands contain the most iodine and the ratio of the iodine content of the small, medium and large glands is approximately as follows:

In the sheep 2:3:7
" " beef 1:1:2
" " hog 3:4:6

The mixed glands arranged according to their iodine content stand about in the following ratio:

Sheep	Beef	Hogs
5	7	9

From whatever standpoint we take we must conclude from the above that the employment of either hogs or beef thyroids for therapeutic purposes would be more rational than the employment of sheep glands, even if small selected sheep glands are employed, thus eliminating the goiterous glands.

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*It is interesting to note that Baumann found very little iodine in pigs' and hogs' thyroid glands; *very* much less than in beef and sheep.

STUDIES ON THE VIRUS OF HOG CHOLERA.

By Walter E. King and Robt. H. Wilson.

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During the course of some experimental work with the "filterable" virus of hog cholera, it was observed that the passage of hog cholera serum through the horse produced various modifications in the virulence of the material. Some four years ago attention was directed to the fact that a relatively short residence of hog cholera serum in the blood of the horse tended to act as an aid in maintaining the virulence of the virus. This phenomenon was noted on several different occasions, but not until two years ago was any definite line of work established to determine the truth of these observations.

The present paper is based partly upon results which were published in September, 1910, in Bulletin 171 of the Kansas State Experiment Station, and partly upon experiments which have been conducted since that publication.

The following experimental data relate to the possible activation of the virus of hog cholera as a result of a relatively short residence of the material in the circulatory system of the horse. The work must be considered as preliminary, and satisfactory explanations of this phenomenon are impossible at the present time. Comments and observations only can be offered.

The strain of virus which was used throughout the work was obtained from Doctors Marshall and Giltner, of the Michigan Experiment Station. This virus, representing the same strain as that in use at several laboratories and obtained originally from Dr. Niles, Ames (Iowa), ordinarily maintained an incubation period of 4 to 7 days and produced typical symptoms and death in 15 days or less.

Use has been made of four experimental horses (Table I), each having been injected intravenously with hog cholera serum at varying intervals. The amount of virus injected each time varied from 20 to 150 c.c., depending upon the condition of the animal and the purpose of the injection. The animals were bled aseptically at intervals of from ½ to 9 hours after injection.

TABLE I.

Treatment of Experimental Horses.

No.	No. of bleeding	Wt. lbs.	Date of injection	Virus injected	Dilution of virus.	Residence of virus in horse.	Lot of serum.
1	1st.	1150	12/30/09	100 c. c.	1 to 344.7	½, 1, 2, 3, 9 hrs.	A
1	2nd.	1105	2/12/10	100 "	1 to 344.7	½ and 9 hrs.	B
2	1st.	800	3/14/10	100 "	1 to 240.3	½ hr.	C
1	3rd.	1150	4/4/10	120 "	1 to 287.2	½ "	D
2	2nd.	800	5/7/10	140 "	1 to 171.7	½ "	E
1	4th.	1150	5/20/10	40 "	1 to 861.8	½ and 1 hr.	F
3	1st.	1050	5/24/10	130 "	1 to 244.2	½ hr.	G
2	3rd.	800	7/2/10	20 "	1 to 1202.0	½ "	H
1	5th.	1150	7/11/10	20 "	1 to 1724.0	½ "	I
1313	1st.	1220	9/23/10	140 "	1 to 264.5	½ "	J
1313	2nd.	1220	10/22/10	140 "	.	¾ "	K
1313	3rd.	1220	11/15/10	33 "		¾ "	L

To test the uniformity of the infectious nature of horse serum virus a series of 18 pigs were each injected with horse serum obtained after ½ to 1 hour's residence of the hog cholera virus in the horse. The results of these tests are given in Table II (cf. p. 369).

TABLE II.

Tests of Horse Serum Virus.

Material injected	No. Hog	Date 1910	Incubat. Period	Results	Autopsy
A. ½ hr. horse ser.	61	1/4	5 days	Died in 11 days	Typical lesions
A. idem	64	1/11	5 "	Died in 11 days	idem
A. "	71	2/8	"	Remained well, natural immune	
A. 1 hr. horse ser.	63	1/4	6 days	Severe reaction, recovered	
A. idem	110	5/21	7 "	Died in 10 days	Slight lesions
B. ½ hr. horse ser.	72	2/19	6 "	Died in 13 days	Typical lesions
B. idem	73	2/19	7 "	Severe reaction, recovered	
C. "	87	3/18	5 "	Died in 13 days	Typical lesions
C. "	88	3/18	5 "	Died in 27 days	idem
D. "	94	4/5	7 "	Died in 13 days	"
D. "	95	4/5	6 "	Died in 13 days	Slight lesions
E. "	107	5/11	8 "	Died in 13 days	idem
F. "	109	5/12	6 "	Died in 9 days	Typical lesions
G. "	126	6/18	10 "	Died in 19 days	idem
G. "	133	6/28	12 "	Died in 19 days	"
J. "	175	9/23	3 "	Died in 11 days	"
K. ¾ hr. horse ser.	186	10/24	5 "	Died in 17 days	"
L. idem	196	11/15	4 "	Severe reaction, died 27 days from other causes	

DILUTION OF HOG CHOLERA VIRUS IN VITRO.

By weighing the horse under observation and estimating the amount of blood contained in his body, it was possible, in the case of each lot of horse serum virus, to estimate the dilution of hog cholera serum *in vivo*, when the animal received intravenously a given quantity of the virus. In order to determine, if possible, whether or not the horse serum virus represented mere dilutions of the original hog cholera virus, corresponding dilutions were made in normal horse blood outside the body of the animal and in physiological salt solution. These dilutions were incubated at body temperature for ½ to 1 hour, depending upon the period of residence of corresponding dilutions of horse serum virus. These tests were made upon hogs in a similar way to those conducted with the corresponding lots of horse serum virus. In all cases, the horse serum virus and corresponding dilutions in the normal horse blood, *in vitro* and in physiological salt solution, were injected in approximate doses of 4 c.c. per 50 pounds weight (Tables III and IV).

TABLE III.

Tests of Normal Horse Blood Dilution, in vitro.

Material injected	No. Hog	Date 1910	Incubation Period	Results	Autopsy
A. ½ hr. dil.	68	2/3	12 days	Died in 20 days	Slight lesions
A. idem	69	2/5	14 "	Slight reaction, recovered	
B. "	79	3/8	8 "	Slight reaction, recovered	
B. "	80	3/8	9 "	Died in 15 days (Not Hog Cholera)	No cholera
C. "	99	4/15	9 "	Slight reaction, recovered	
C. "	100	4/15	10 "	Died in 17 days	Typical lesions
E. "	113	5/27		No reaction	
F. "	132	6/23	18 "	Died in 21 days	Slight lesions
G. "	134	6/30		No reaction	
J. "	178	10/5	5 "	Mild reaction	
L. ¾ hr. dil.	199	11/29	9 "	Died in 14 days	Typical lesions

TABLE IV.

Tests of physiological salt dilutions.

Material injected	No. hog	Date 1910	Incubation Period	Results	Autopsy
A. 2 hr. dil.	70	2/5	15 days	Slight reaction, recovered	
B. idem	81	3/8	18 "	Recovered	
B. ½ hr. dil.	75	2/28	14 "	Died in 22 days	Slight lesions
C. idem	93	3/28	14 "	Died in 18 days	Typical lesions
D. "	108	5/11	8 "	Died in 10 days	idem
E. "	112	5/26	9 "	Died in 23 days	"
F. "	118	6/8	17 "	Mild reaction, recovered	
G. "	131	6/18	18 "	Died in 23 days	Slight lesions
J. "	176	9/29	7 "	Died in 19 days	Typical lesions
L. ¾ hr. dil.	198	11/29	Temp. reac. 8 days	No clinical reaction	

The results of the completed tests of hog cholera virus diluted in physiological salt solution may be used in indicating something as to the minimum fatal dose of the filterable hog cholera virus (Table V).

TABLE V.

Attempted determination of Minimum Fatal Dose of Hog Cholera Virus, Dilution Physiological Salt Solution.

Hog No.	Date 1910	Virus obtained from	Dilution in physiol. salt	Approximate amount injected	Incubation period	Result	Autopsy
112	5/26	Hog 97	1 c. c. virus 172 c.c. physiol. salt	1/43 C. C.	9 days	Died in 23 days	Typical lesions
131	6/18	" 105	1 c. c. virus 244 c.c. physiol. salt	1/61 "	18 "	Died in 23 days	Slight lesions
176	9/29	"	1 c. c. virus 244 c.c. physiol. salt	1/66 "	7 "	Died in 19 days	Typical lesions
108	5/11	" 89	1 c. c. virus 287 c.c. physiol. salt	1/72 "	8 "	Died in 10 days	idem
93	3/26	" 72	1 c. c. virus 320 c.c. physiol. salt	1/80 "	14 "	Died in 18 days	idem
75	2/28	Hog 64	1 c. c. virus 344 c.c. physiol. salt	1/86 c.c.	14 days	Died in 22 days	Slight lesions
118	6/8	" 97	1 c. c. virus 862 c.c. physiol. salt	1/125 "	17 "	Mild reaction, recov.	
198	11/29	"	1 c. c. virus 1113 c.c. physiol. salt	[illegible] "	Temp. reaction 8 days	No clinical react.	
136	7/6	" 122	1 c. c. virus 1202 c.c. physiol. salt	1/300 "	No reaction		
138	7/14	" 122	1 c. c. virus 1724 c.c. physiol. salt	[illegible] "	id.		

The summarized findings of the above tests of horse serum virus, corresponding dilutions in normal horse blood and corresponding dilutions in physiological salt solution are arranged in Table VI.

TABLE VI.

Summary, Comparative results of dilution tests. Manner of dilution of virus.

	Horse serum virus	Corresponding dilutions in normal horse blood incubated half hour at body temperature	Corresponding dilutions in physiological salt solution, incubated half hour at body temperature
No. of hogs in series	18	11	10
Average incubation period	6.3 days	10.3 days	12.8 days
No. of hogs recovered	3 (severe reactions)	6	4
Percentage of hogs which died	82.5%	54.5%	60%
Average duration of disease	14.9 days	17.4 days	19.1 days
General character of disease	Acute	Chronic and Slight	More chronic and erratic
General character of lesions	Good	Slight, not marked	Good

TOXIC ACTION OF HOG CHOLERA VIRUS UPON THE HORSE.

The amount of filterable hog cholera virus which may safely be injected into the horse at any one time, is usually limited to about 150 c.c. Each succeeding experiment appears to make more evident the fact that the hog cholera serum causes a marked toxic action on the horse. While the symptoms which follow the injection of the virus are similar in nature to those produced by the intravenous injection of normal hog serum, yet the former are much more pronounced. These symptoms are usually characterized immediately after treatment by increased painful respiration and accelerated heart action; the appearance of a profuse, clammy sweat; active peristalsis and rarely epistaxis. Approximately ½ hour after the injection, the heart becomes depressed, the pulse sometimes almost obliterated, the eye dull and listless and conjunctiva congested. The peristalsis usually becomes violent and frequent, watery evacuations appear. After one to two hours, especially when the horse is relieved of a quantity of blood, the symptoms gradually subside and in ten hours the animal may become normal in condition, with the exception of a greater or less degree of exhaustion.

The injection of similar quantities of normal hog serum usually produces slightly accelerated heart action and respiration, and rarely marked peristalsis and watery evacuations. The symptoms are much less pronounced than those following the injection of hog cholera serum and usually disappear in a short time.

In addition to the observations following the injection of some ten horses with hog cholera serum, some comparative results have been obtained relative to the effects of hog cholera serum and normal hog serum upon the horse.

One animal was injected first with 140 c.c. normal hog serum and 19 days after the first injection the animal received 130 c.c. of hog cholera serum. To eliminate inaccurate results due to possible sensitization of the horse to the foreign serum before the injection of the virus, another animal, horse No. 2, was injected first with 3 c.c., then at intervals of 3, 10, 4, 7, 6 and 8 days with 6, 17, 30, 42, 68 and 75 c.c. of hog cholera serum, respectively. Eleven days after the last injection of 75 c.c. of hog cholera serum this horse received intrajugularly 85 c.c. of normal hog serum. The clinical symptoms were carefully noted after each of the above injections.

At the present time two horses are receiving parallel treatments with normal and hog cholera serum.

The summarized results of the above comparative tests are presented in Tables VII and VIII.

TABLE VII.

Comparative results following the intrajugular injection of the Normal and Hog Cholera Serum.

Horse No.	Wgt.	Date 1908	Material injected	Am't.	Symptoms noted after injection
4	1300	7/31	Normal Hog Serum	140 c.c.	Symptoms not pronounced. Heart respiration slightly accelerated
4	1300	8/19	Hog Cholera Serum	130 "	Violent symptoms, 10 watery evacuations in 20 minutes. Decubitus.
2	1000	7/3	idem	30 "	No symptoms
2	1000	7/7	"	6 "	" "
2	1000	7/17	"	17 "	" "
2	1000	7/21	"	30 "	Slight symptoms
2	1000	7/28	"	42 "	Disturbed heart action, resp.
2	1000	8/3	"	68 "	Disturbed heart action, resp. and active peristalsis present
2	1000	8/11	"	75 "	Violent symptoms. Copious perspiration, symptoms very pronounced for 3 hours
2	1000	8/22	Normal Hog Serum	85 "	Horse eating grass 5 min. after treatment. Symptoms slight

TABLE VIII.

Parallel injection, Hog Cholera Serum and Normal.

Horse No.	Wgt.	Date	Material injected	Am't.	Symptoms noted after injection
1313	1220	9/22	Hog Cholera Serum	140 c.c.	Marked symptoms, 3 watery evacuations in 5 minutes
1314	870	10/7	Normal Hog Serum	140 "	Respiration dist. pronounced. Heart slightly accelerated. Horse broke loose, fell and became fastened in stocks. Nervousness modified the results. No abnormal defecations.
1313	1220	10/22	Hog Cholera Serum	140 "	Very pronounced. Restoratives necessary
1314	870	11/15	Normal Hog Serum	140 "	Similar but less marked respiratory changes than preceding
1313	1220	11/15	Virus	33 "	Accelerated heart resp. increased
1314	870	11/29	Normal Serum	33 "	Slight symptoms which subsided in 10 minutes.

CHANGES IN THE BLOOD OF THE HORSE FOLLOWING THE INJECTION OF HOG CHOLERA VIRUS.

A study of the blood of the horse before and after injection with hog cholera virus (Tables IX and X) shows a decrease of red corpuscles and a marked leucocytosis. The specific gravity of the blood is also decreased after the injection.

From the very limited array of results (Table XI) it is indicated that the blood changes are less pronounced after the injection of Normal Hog Serum as compared with those after the injection of Hog Cholera Serum.

TABLE IX.

Comparative average Results from Study of the Blood, Horse 1, before and after three intravenous injections with virus.

	Before Injection	After Injection
No. erythrocytes	7 460 000	6 204 000
No. leucocytes	11 440	16 150
Per cent lymphocytes	41.2	49.2
Per cent large mononuclears	2.5	0.9
Per cent polynuclears	49.3	44.2
Per cent eosinophiles	8.1	5.0
Per cent transitional forms	0.65	—
Per cent mast cells	0.12	0.2
Per cent hemoglobin	87.0	84.0
Specific gravity	1060.0	1056.0
Time of coagulation	3 min. 10 sec.	3 min. 8 sec.

TABLE X.

Comparative Average Results from Study of the blood, Horse 2, before and after two intravenous injections with virus

	Before Injection	After Injection
No. erythrocytes	8 450 000	6 750 000
No. leucocytes	11 075	15 300
Per cent lymphocytes	51.8	38.6
Per cent large mononuclears	2.2	1.9
Per cent polynuclears	42.9	53.0
Per cent eosinophiles	3.4	5.4
Per cent transitional forms	0.5	0.4
Per cent mast cells	0.27	0.6
Per cent hemoglobin	95.0	89.0
Specific gravity	1060.0	1057.0
Time of coagulation	3 min. 23 sec.	3 min. 10 sec.

TABLE XI.

Comparative average results from the study of the blood of horse 1313 and horse 1314 before and ½ to ¾ hours after the second intravenous injection of each with like volumes of hog cholera serum and normal hog serum, respectively.

	Hog Cholera Serum		Normal Hog Serum	
	Horse 1313		Horse 1314	
	Before inj.	After	Before	After
No. erythrocytes	5 000 000	3 000 000	5 000 000	5 036 000
No. leucocytes	14 000	10 000	9 000	10 500
Per cent lymphocytes	50.3	53.4	41.3	67.0
Per cent large mononuclears	0.7	1.2	2.8	2.1
Per cent polynuclears	42.8	42.0	49.0	26.4
Per cent eosinophiles	3.8	3.0	6.7	4.3
Per cent transitional forms	0.4	0.1	0	0
Per cent mast cells	0	0	0	0
Per cent hemoglobin	95.0	85.0	95.0	95.0
Specific gravity	1060.0	1056.0	1060.0	1058.0
Time of çoagulation	2 min. 40 sec.	3 min.	2 min.	4 min.

A residence of hog cholera virus in the blood of the horse for a period of ½ to 1 hour appears to cause an activation of the virus for the following reasons:

(1) In a series of 18 pigs, ½ to 1 hour horse serum virus produced death in 82.5 per cent of the animals, with an average incubation period of 6.3 days and an average period of duration of the disease of 14.9 days. In comparison with the above, corresponding dilutions with the same lots of virus in normal horse blood *in vitro*, and in sterile physiological salt solution, in series of 11 and 10 pigs, respectively, produced death in 51.4 and 60 per cent of the animals, with average incubation periods of 10.3 and 12.8 days and average disease durations of 17.1 and 19.1 days, respectively.

(2) Horse serum virus, when injected into the healthy hog, almost invariably produces symptoms after a relatively short incubation period, as compared with the injection into the healthy hog of a similar quantity of the same dilution of virus in normal horse blood, *in vitro*, physiological salt solution or even the undiluted original hog cholera serum.

(3) The minimum fatal dose of virulent serum, as represented by a dilution of the virus in physiological salt solution, does not appear to indicate the minimum fatal dose when the dilution is maintained in the form of horse serum virus.

(4) Hog cholera serum injected intravenously into horses causes more pronounced toxic symptoms than do similar treatments with normal hog serum.

(5) The changes in the blood of the horse following the injection of hog cholera serum are possibly greater than those resulting from the injection of normal hog serum.

Very little can be offered relative to the definite action upon the virus, as evidenced by this phenomenon of so-called activation. No satisfactory explanation can be given until we understand more thoroughly the nature of the filterable virus of hog cholera. The period of residence of hog cholera serum in the circulatory system of the horse necessary to cause this so-called activation of the virus is relatively short in duration. Because of this it seems improbable that the process involves a multiplication of the theoretical, invisible organisms. If it is assumed that the activation does not materially involve the living infectious agent, then it appears logical to ask whether this phenomenon can be associated with possible toxin constituents of the filterable virus.

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ON THE CULTIVATION OF THE TREPONEMA PALLIDUM (SPIROCHAETA PALLIDA).*

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Attempts to cultivate the treponema pallidum in artificial media followed closely on the discovery that this organism is the cause of syphilis. Volpino and Fontana[1] reported in 1906 on the cultivation of the organism in serum-agar. The observations of Volpino and Fontana were corroborated by Schereschewsky[2] in 1909. By inserting deep into coagulated horse serum small pieces of human tissue from syphilitic lesions, he obtained impure cultures. The horse serum was liquefied around the tissue, and in this he was able to find the spirochætes. These cultures he was able to transfer. Mühlens,[3] in 1910, made use of the same medium, as did also Hoffmann,[4] in 1911, and both were able to obtain cultures of the organism. These cultures also were contaminated with bacteria introduced with the tissue used for starting the cultures. For the purpose of purifying these cultures they employed serum-agar. The attempts of these investigators to produce syphilitic lesions through the injection of cultivated spirochætes failed for a time, probably because the dose employed was too small. Both Schereschewsky and Mühlens concluded that the strains which they had cultivated were non-pathogenic.

Brückner and Galasesco[5] in 1910, as well as Sowade[6] in 1911, claimed to have succeeded in producing syphilitic lesions in rabbits by the injection of large quantities of impure cultures grown on coagulated horse serum and ascitic fluid media still containing the original syphilitic tissue. The failure to grow again the spirochætes from the testicular lesions produced lays the results claimed by these investigators somewhat open to question.

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[1] *Deutsch. med. Wchnschr.*, 1909, 35, p. 835.

[2] *Ibid.*, p. 1260.

[3] *Ibid.*, p. 1261; *Klin. Jarb.*, 1910, 23, p. 339.

[4] *Zeitsch. f. Hyg. u. Infekt.*, 1911, 68, p. 27.

[5] *Compt. rend soc d. Biol.*, 1910, 68, p. 684.

[6] *Deutsch. med. Wchnschr.*, 1911, 37, p. 682.

Noguchi[7] in 1911 published a method for the pure cultivation of pathogenic treponema pallidum. Instead of attempting to obtain a pure culture of the organism through cultivation in artificial media, he first transplanted the human syphilitic tissue into the testicles of rabbits. This procedure, if carefully done, so as to avoid accidental contamination, will in a few passages through rabbits clear the strain of spirochætes from the accompanying infection so that in a comparatively short time lesions may be produced in rabbits which contain the organisms in pure form. These Noguchi used for cultivation experiments. After many unsuccessful attempts with various media he found that inactivated serum-water was most suitable for the purpose. In order to obtain strict anaerobiosis he placed a small piece of normal rabbit's tissue, either kidney or testis, at the bottom of the medium. He found it necessary to place the culture under hydrogen with pyrogallic acid and potassium hydroxid. The spirochætes thus grown could be gradually transferred to solid media, *i.e.*, ascitic-agar plus tissue. These cultures were pathogenic for rabbits. This announcement of the successful inoculation of rabbits with the treponema pallidum grown in culture was followed, within a month, by that of Hoffmann[8] and Schereschewsky,[9] who had succeeded also in producing syphilitic lesions in rabbits through the injection of pure cultures of this organism.

Since then numerous investigators have taken up the cultivation and study of the treponema pallidum. Aside from those already mentioned, Leuriaux et v. Geets,[10] Tomaszewski,[11] Sowade,[12] Arnheim,[13] and others have succeeded in growing this organism.

In this country Noguchi seems to be the only investigator who has reported on the cultivation of the treponema pallidum. The reason for this lack of confirmatory work may lie in the rather difficult method which he uses.

The media described by Noguchi as most suitable for the cultivation of the treponema pallidum offer many difficulties and demand extraordinary care. The solid media have to be prepared fresh for each transplantation, for it is necessary that the tissue, placed in the bottom of the tube, be fresh. Furthermore, the use of neutral oil on the top of the media increases the difficulty of making examinations from time to time. Noguchi also pointed out that not all lots of ascitic fluid are suitable for the ascitic-agar. Having tried many without success, until I finally found one, I faced the same difficulty again after that was used up. For these reasons it seemed worth while to test also the methods used by other investigators, since these methods are simpler and the materials used for the making of the media more constant and easier to obtain.

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[7] *Jour. Exper. Med.*, 1911, 2, 14, p. 99; *Munchen. med. Wchnschr.*, 1911, 29, p. 1550.
[8] *Munchen. deutsch med. Wchnschr.*, 1911, 34, p. 1546.
[9] *Ibid.*, 1911, 39, p. 1798; *Ibid.*, 1912, 38, p. 1355.
[10] *Centralbl. f. Bakt.*, 1912, 61, p. 684.
[11] *Berl. klin. Wchnschr.*, 1912, 17, p. 792.
[12] *Deutsch. med. Wchnschr.*, 1912, 17, p. 797.
[13] *Ibid.*, 1912, 20, p. 954.

Probably the fact that the investigators abroad started their cultures directly from the syphilitic lesion of man, whereas Noguchi at first made use of material obtained by passing the treponema pallidum through rabbits, brought about a divergence in the interpretation of the cultural characteristics of this organism and cast doubt upon the genuineness of the cultures in the possession of some of the investigators. Thus Noguchi maintains that the following conditions are essential to the growth of the treponema pallidum: (1) the presence of suitable fresh sterile tissue in serum-water, (2) strict anaerobiosis, (3) a slightly alkaline reaction as furnished by the serum and tissue, and (4) a temperature of about 35° to 37° C. Noguchi further states: "The presence of agar or gelatin seems to interfere with the successful growth of the first generation of the spirochæta." Arnheim, cultivating the treponema pallidum directly from the human syphilitic lesion, plants his material into serum-agar without tissue. Nakano[14] cultivates the treponema pallidum in sterile horse serum without the addition of tissue and the strict anaerobiosis, using a rubber stopper only to exclude air. Schereschewsky and Sowade make use of the same technic as Arnheim. Their cultures were obtained without the addition of sterile tissue, or strict anaerobiosis. Furthermore, Schereschewsky[15] observed that the treponema pallidum will grow well at a temperature of 40° C. It is also interesting to note that Noguchi has been able to grow the treponema pallidum without the hydrogen, simply placing a layer of sterile neutral oil on top of his media. This diversity of conditions under which these cultures of the treponema pallidum were grown may be due to the fact that on the one hand the cultures were started from material which has been purified by the passage through rabbits, while on the other hand the cultures were obtained directly from human lesions rich in the treponema.

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[14] *Deutsch. med. Wchnschr.*, 1912, 28, p. 1334.

[15] *Ibid.*, 1912, 28, p. 1335.

My attempts to cultivate this organism were begun in the fall of 1911, and efforts were first directed to the inoculation of rabbits in order to obtain material rich in the treponema and possibly sufficiently pure from banal bacteria to attempt cultivation. The unpleasant odor of the cultures, as well as the liquefaction of the culture medium, described as characteristic of the treponema culture by some of the earlier investigators, seemed to me to be due to the concurrent infection and not due to the treponema pallidum. Another reason why I transplanted first into rabbits before attempting to inoculate culture media, was the fact that the opportunity for obtaining syphilitic tissue containing the treponema is relatively limited. It was necessary to use the serum which was drawn from the primary sore by means of a specially devised cup and suction pump. The small amount of serum thus obtained was employed for diagnostic purposes as well as for the inoculation of rabbits.

The apparatus for obtaining the serum consists of a test tube drawn to a fine point, with a side arm for the attachment of the rubber tube, which in turn is connected to a small suction pump. The tube has a fairly large lip, which allows it to fit snugly on the tissue surrounding the primary lesion. I use cupping tubes varying in diameter to accommodate chancres of different sizes. The pump is manipulated by means of a crank and has a screw on its lower end, which when loosened will allow air to enter. This permits of releasing the vacuum in the cupping tube, and does away with any pain which the removal of the tube might cause (Fig. 1).

The inoculation of the rabbit is made with a glass hypodermic syringe in such a way that a small depot of the serum is created in the testicle of the rabbit. With this method I have obtained two strains of the treponema pallidum. The period of incubation following the injection of serum is from eight to ten weeks. The lesions produced at that time were the size of a pea and contained in both instances numerous actively motile spirochætes. Since then I have succeeded in growing four strains of the treponema pallidum. Of these strains, the first, second, and fourth were obtained through the implantation of small pieces of genital chancre tissue into the testicles of rabbits, the third strain was obtained by the injection into the testicle of a rabbit of a small quantity of serum from another initial lesion. The first strain obtained by the injection of serum obtained by cupping was lost, because the rabbits inoculated in the second generation died of an intercurrent infection. Strains one and two were grown from material supplied by Dr. F. M. Hartsock, whose generous assistance at this stage of the work I gratefully acknowledge.

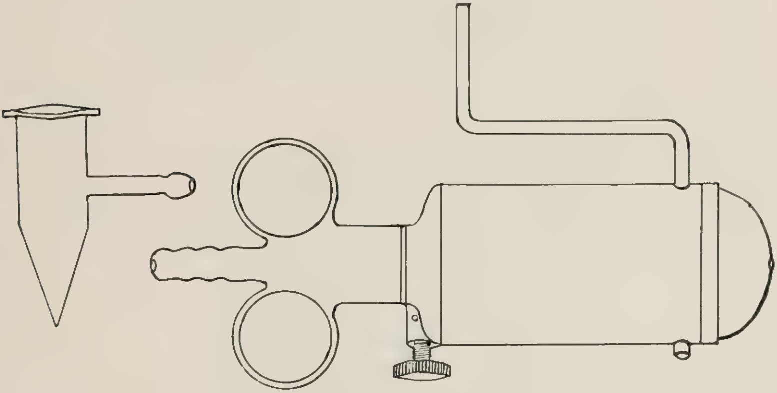

Fig. 1.—Apparatus for obtaining serum.

The transplantations from rabbit to rabbit are carried out as follows: A rabbit with a good-sized unbroken testicular lesion is anesthetized; the lower portion of the abdomen as well as the scrotal region is shaved and sterilized with germicidal soap and alcohol. The rabbit is covered, except the field of operation. The testicle is then removed under aseptic precautions and placed in a petri dish, which is kept warm. To prevent drying, a few drops of sterile broth are added. When the operation is completed the testis is cut open and the white glistening gumma is cut free from the unaffected part of the testis and placed in another petri dish containing a small amount of broth. The gumma is then cut into small pieces, and a small quantity of the tissue scraping is examined under the dark field to determine the presence of the treponema pallidum. A few smears are stained with methylene blue to determine whether the tissue to be used is free from other organisms. To make doubly sure tubes of plain broth and agar are inoculated. The rabbits are then inoculated by means of a small trocar. The inoculation with the trocar has proven more successful than the injection of ground-up tissue. The organisms are not injured to such an extent when solid pieces are introduced as when the material is subjected to a process of grinding and admixing with a diluent. (I also wish to express my thanks to Drs. Keene, Varney, and Wollenberg for aiding me in obtaining material for this work.) The area of inoculation is cleaned with 70 per cent alcohol and the instruments are boiled for the inoculations of each rabbit. This procedure has the two advantages which Noguchi has pointed out: (1) it enables one to obtain large quantities of spirochæte-containing material at almost any time; (2) the spirochætes are freed from concurrent microörganisms.

The lesions observed in rabbits vary considerably in severity, general appearance, and location. The most common lesion observed in my work of transplantation of syphilitic tissue in rabbits is a syphilitic orchitis, which affects part of the testis only. The scrotum shows no ulceration or lesion or orchitis. The testis is of uniform consistency, except the portion involved by the circumscribed gumma. The lesion is pure glistening white. The fluid, which adheres to the knife on cutting into the lesion, is of a stringy mucoid character, and contains numerous spirochætes. Such an orchitis may involve the entire testis. The testicle involved is considerably larger than normal.

The second form of syphilitic lesion appears in the form of small nodules or thickenings, which are situated in the tunica. This form of lesion may be circumscribed or it may involve almost the entire tunica. The testis in this case appears small. These nodules and thickenings contain numerous spirochætes.

The third form is the ulcer. This varies in size from a few millimeters to a centimeter and even more in diameter. The edges are indurated and the ulcer has the characteristic punched-out appearance. In this form the spirochætes may be found in the indurated area around the ulcer. The size of the testis is usually not affected by this form of syphilitic lesion.

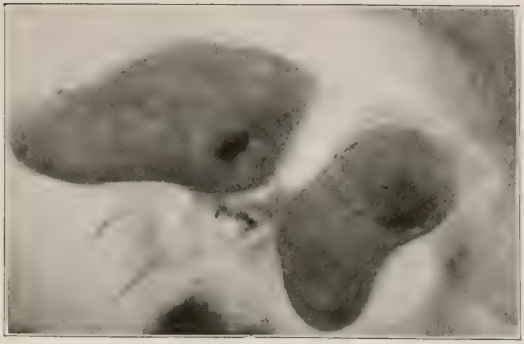

FIG. 2.—Syphilitic orchitis in a rabbit, fifth generation of strain one.

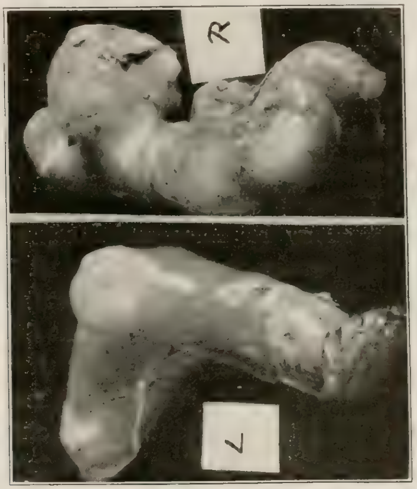

FIG. 3.—The testes of the same rabbit. The lesions may be recognized as white protruding masses.

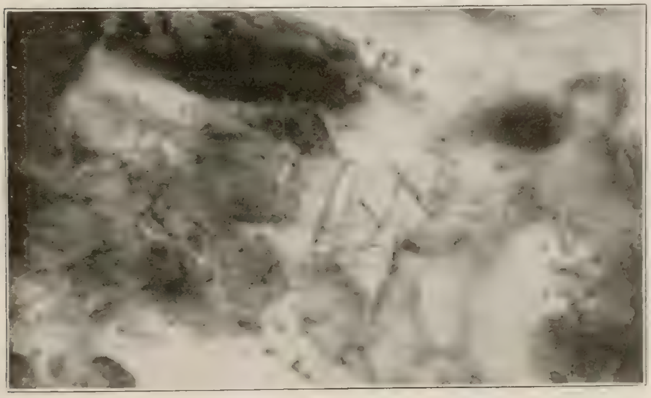

FIG. 4.—Testicular lesion of the rabbit, impregnated with silver, according to Levaditi.

These varieties of lesions are possibly due to the technic of inoculation, since the tissue frequently adheres to the trocar, is pulled out toward the scrotum, and so is lodged in the tunica, or scrotal skin. The forms described are localized conditions, which in rabbits tend to heal. We have never observed generalized syphilis nor any secondary manifestations.

Soon after the strains 1 and 2 had been passed through rabbits a number of times, attempts were made to cultivate the organism. The cultivation of the treponema pallidum demands patience until a difficult technic is fairly well mastered. It is intended to describe the methods employed in detail so that any one having the necessary laboratory facilities may grow the organism.

The plan followed in my experiments has been to determine whether the treponema pallidum can be grown both according to the methods of Schereschewsky, Mühlens, Hoffmann, and according to the method described by Noguchi, thus reconciling the contradictory views regarding the genuineness of the cultures claimed by some of the investigators.

The media employed by Mühlens and Hoffmann are coagulated horse serum and serum-agar. Sterile test tubes are filled with fresh horse serum free from any preservative, and these are sterilized in a water bath for one hour at 60° C. for three successive days. On the third day the temperature is allowed to rise gradually to 70° C. The horse serum will become of a jelly-like consistency and will be transparent. About one-half the tubes are removed from the water bath, when the serum column will not flow if the tube is held in a horizontal position. This soft horse serum medium is then placed in the incubator for three days, and when found sterile the tubes are capped to prevent evaporation.[16] The remainder of the tubes are allowed to reach a firmer consistency without, however, losing the transparency. This set of media is also tested for sterility, and then capped. The first lot of tubes are used for the initial step of cultivating the treponema pallidum. The growth of this organism is often hindered if too dense media are employed.

The horse serum used for the serum-agar is put into sterile test tubes, so that the tube is about one-third filled. The serum

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[16] The horse serum medium undergoes partial autolysis during this time and is preferable to horse serum not incubated.

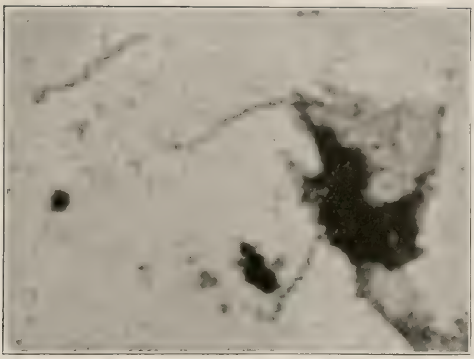

FIG. 5.—Cover glass smear from testicular lesion of a rabbit stained with Giemsa's method.

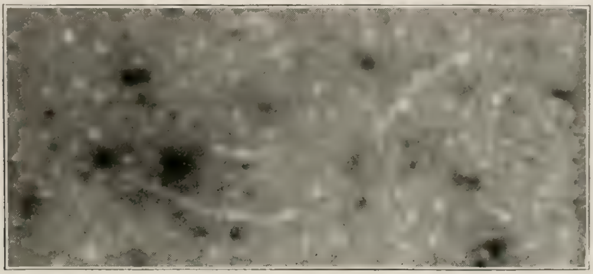

FIG. 6.—Treponema pallidum from a culture. An india ink preparation.

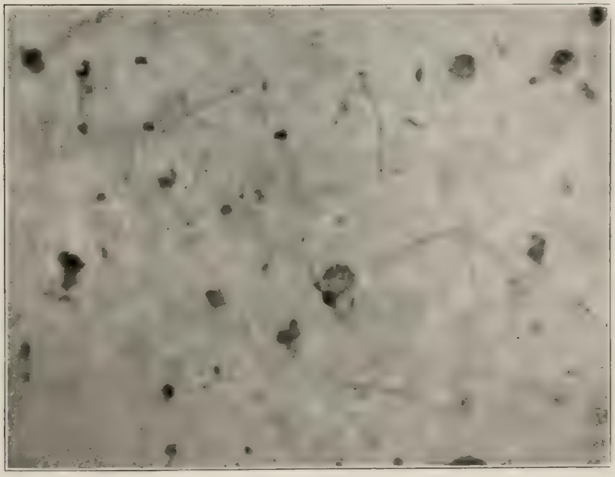

FIG. 7.—Treponema pallidum from a culture. Giemsa stain.

is then sterilized by placing it in a water bath for one hour at 55° C. for three successive days. A 3 per cent agar containing 0.5 per cent glucose is then melted, and when cooled to 50° C. the tubes containing the heated serum are filled with melted agar equal to the amount of serum they already contain. It is advantageous to have sufficient assistance so that the tubes can be rolled to mix the agar and serum thoroughly. While the serum-agar in the test tubes is still liquid, the tubes are placed again in the water bath at 55° C., which permits the media to clear. This requires about two hours. The medium will then be of a clear golden color and may be permitted to harden. The test tubes used are from 13 to 15 cm. in length. The serum-water plus tissue medium, as well as the ascitic agar plus tissue medium used by Noguchi, is placed into tubes 20 cm. high. Such tubes are too long for the ordinary desiccators in laboratory use. To overcome this difficulty I have made use of large graduates with glass stoppers, with the mouth large enough to permit the passage of a large test tube. A rubber stopper with two holes is used instead of the glass stopper, and two glass stop-cocks are passed through the holes of the rubber stopper. The pyrogallic acid is first placed in the graduate, then the inoculated tubes, and after that the rubber stopper with one of the glass cocks in place. To this glass cock is attached a rubber tubing long enough to reach to the bottom of the graduate. The second glass cock is then pushed through the rubber stopper. This causes the stopper to fit tightly. The same process is followed when desiccators are used for the cultivation of the treponema pallidum in coagulated horse serum and serum-agar media.

Although Noguchi found it necessary to begin the cultivation of the treponema pallidum in heated horse serum plus tissue, the organism can be grown in coagulated horse serum, either by inserting a small piece of tissue containing the organisms, or by grinding up the tissue, diluting it with broth, and making inoculations into this medium by means of a capillary pipette. To inoculate with tissue it is best to use it in as fresh condition as possible. The tissue is divided into small pieces the size of a split pea. With a sterile platinum wire the tissue is placed deep in the medium between the wall of the tube and the medium. The introduction of air bubbles must be avoided. The consistency of the media permits this method of inoculation without tearing. It has not been found necessary to place neutral oil on the surface of the media. The inoculated tubes are placed in a desiccator containing pyrogallic acid, and the desiccator is then exhausted and hydrogen passed through it until a test tube held to the outlet tube contains hydrogen. Suction is again applied to produce a vacuum in the desiccator and a strong solution of potassium hydroxid is run in through the outlet tube.

The alkaline pyrogallol solution is used in such proportion that one volume of a 22 per cent aqueous solution of pyrogallol is mixed with five or six times as much potassium hydroxid solution (3:2). At a temperature of 15° C. or higher the oxygen is quickly absorbed and a pyrogallol solution of the above concentration will not evolve carbon monoxid during the absorption.[17]

Some investigators use rubber stoppers and dispense with the hydrogen, pyrogallic acid, and potassium hydroxid. The method employed by myself has seemed preferable because the surface of the cultures will be found entirely sterile, since the cotton plugs can be flamed, and on subsequent examination of the cultures one can obtain the media containing the spirochætes near the tissue, or liquid inoculated by means of a sterile pipette. The cultures should be examined about one week after inoculation, when one will find numerous characteristically motile spirochætes if the inoculation has been successful. The work requires perseverance, and not all strains of the treponema pallidum will lend themselves to cultivation.

The pipette used is about 25 cm. long, drawn out, the upper end of such diameter that the rubber nipple will fit snugly. The lower end of the pipette is sealed and a hole is blown into it on the side, close to the lower end. This arrangement will prevent the media from rising into the lumen of the pipette when inoculations are made, or when it is desired to remove some of the culture for examination (Fig. 8).

The growing treponema pallidum may be observed to radiate from the tissue implanted in the coagulated horse serum in the form of a very faint cloud. The colonies are not sharply defined, nor distinct in their outline. The object of implanting the tissue near the wall of the test tube is to permit the growing treponema to penetrate into the medium. Thus one is enabled to obtain pure cultures. The cultures growing in the ascitic-agar plus tissue grow deeply at first, and gradually the growth extends in a fine haze to within an inch of the surface of the medium. The coloring of the ascitic-agar by the hemoglobin from the tissue makes it very difficult to detect the colonies, and we have had tubes which, although appearing on inspection to be negative, were found to contain the organism when allowed to stand in the incubator for several weeks longer. Hazy colonies could then be detected above the reddened portion of the media. In the comparatively short time since this work was undertaken I have succeeded in growing one strain pure for four generations, and this strain was grown both in serum-water plus tissue and oil; ascitic agar plus tissue and oil in a hydrogen atmosphere, as well as in horse serum media, as described above and used by Mühlens, Hoffmann, and others. I have also succeeded in obtaining a typical syphilitic lesion in the testis of one of the two rabbits inoculated. The lesion was a circumscribed orchitis, not broken through the skin. This lesion, which was noticed five weeks after inoculation, was about the size of a cherry stone, and showed numerous actively motile spirochætes under the dark field. This culture was devoid of any unpleasant odor, whether grown in coagulated horse serum, serum-agar, serum-water plus tissue, or ascitic-agar plus tissue. Cultivation experiments with the other strains are in progress.

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[17] Treadwell and Hall, *Analytical Chemistry*, Vol. 2, p. 686.

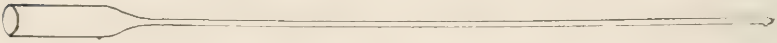

FIG. 8.—Pipette used for inoculation.

In conclusion, it may be pointed out that the treponema pallidum of Schaudinn may be grown on various media and under varying conditions of anaerobiosis.

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STUDIES ON THE GONOCOCCUS.* I.

CARL C. WARDEN.

(From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.)

The present study is based on the study of 15 strains of *Micrococcus gonorrhea* (Neisser) obtained from the urethral discharges of acute cases of gonorrhea in men. All primary cultures were made on ascites fluid agar or on rabbit blood agar.

CULTURE MEDIA.

Body fluid media.—For the successful cultivation of the gonococcus slightly acid (+0.7 to 1.5) media, containing preferably human body fluids, such as ascitic or hydrocele fluid, blood or blood serum, are essential. I have been unable to substantiate the claims of Thallman,[1] Paldrock, and others as to the utility of simple media, urinary and otherwise. They are of no value whatever. Hydrocele or ascitic broth is of considerable value in the detection of contaminating organisms and in the preservation of cultures of gonococci for considerable periods of time. Contrary to the statements of some observers, the gonococci grow only on the surface of this medium. The sediment consists wholly of dead organisms and their débris. Blood agar, prepared both by admixture of blood before the agar solidifies and by smearing blood over the solid agar surface, is valuable, but less so than the ascitic or hydrocele agar. It is well to emphasize the importance of a slightly acid reaction in all media. It has been pointed out that human blood serum, hydrocele, and ascitic fluids may be alkaline to litmus and acid to phenolphthalein. In general, an acidity of +0.7 to 1.5 (10 c.c. medium requires 0.07 to 0.10 $n/10$ NaOH) will be found most satisfactory. It is only partly true that almost any alkaline medium is suitable for the growth of gonococci. Occasionally, abundant growths may be obtained on simple alkaline media, but such growths always show exces-

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[1] *Centralbl. f. Bakt.*, 1900, i, 27, p. 828.

sive autolysis and precarious stability, while more often there results no growth at all.

When blood is streaked on the agar surface the individual colonies present a characteristic and unmistakable appearance. In 24 hours they are less than pinhead size, gray-white, opaque, irregularly round, slightly convex, and with a ×8 hand lens they show a crumbly consistence and a finely stellate or radiate arrangement, the red blood having settled into the radiations like red spokes in a wheel. Very minute concentric striæ or lamellæ may be observed with a higher power. In examining such colonies it is well to hold the tubes up to a strong light and examine the colonies from the back. The blood should not be so thick in the medium as to prevent translucence. The separate colonies are easily picked off *in toto* with a fine platinum wire. Such colonies may attain ¼-inch size in 48 hours. Old colonies take on a glistening silvery sheen.

Artificial serum medium.—The effort to find some medium which, free from body fluid, would support life in gonococci led me to try innumerable combinations, with the result that one was hit upon which promised gratifying results. This consisted of a modified Ringer's solution, the formula for which follows:

Sodium chlorid	1.08
Potassium chlorid	0.045
Calcium chlorid	0.025
Sodium bicarbonate	0.020
Agar	0.250
Nutrient broth	20.00
Distilled water	100.00

The salts and agar are dissolved in the water by the aid of heat until the fluid shows a translucent ground glass appearance. To this is added the broth, and the whole is filtered while hot through gauze or cotton into test-tubes or flasks. One sterilization in the autoclave is sufficient. The medium does not stand repeated Arnold sterilization as the agar is apt to be thrown out of suspension. When cool the medium is semisolid and has a translucent silvery appearance.

The limitations of this medium are that not all strains of gonococci will grow on it, nor is it easy to prepare, nor does it lend itself readily to centrifugation. The medium will, however, support all strains of gonococci, if there be placed on its surface a few loopfuls or a tiny drop of sterile human blood. The gonococci then thrive, while contaminating cocci and bacteria of all kinds except the subtilis bacillus settle to the bottom of the tubes or flasks in trailing white, comet-like or stalactite-like festoons. The medium supports life in gonococci longer than any medium known to me, successful transplantations having been made after 100 days. It is also valuable for isolation of pure strains. It is by no means uncommon that individual gonococcus colonies on solid media contain contaminating organisms, so that pure strains are difficult to procure. Indeed, every one who has worked long on the gonococcus knows this peculiarity and appreciates the difficulty of obtaining pure cultures. Not infrequently the procedure requires a struggle of weeks or months.

Beer-wort and media containing alcohol are of no value.

It is the observation of many bacteriologists that the gonococcus produces acid in dextrose media, but does not change saccharose, lactose, inulin, levulose, galactose, or mannite. Using ascitic broth to which the various carbohydrates were added in varying proportions and of which the reactions varied from slightly alkaline to +2.0, this predilection was confirmed. Titration of the medium after incubation at 37° C. for 14 days invariably showed increased acidity in the tubes containing glucose, but no changes with saccharose, inulin, or mannite. One strain out of 15 showed slight changes in tubes containing galactose.

The conclusions of Watabiki[1] were not confirmed, although horse serum which that author used as the diluent for his fluid media was not employed.

Media containing organic substances.—To ordinary nutrient agar and nutrient broth were added varying proportions of such animal substances as powdered mucin, fibrin, ovary, testicle, thyroid, pituitary, and lymph gland. Of media so prepared those containing ovarian and the testicular substance one to two per cent, slightly acid in reaction, proved excellent, while the others gave indifferent results. Vegetable substances, such as yeast nuclein, Irish moss, tragacanth, and acacia, were used in a similar manner. Of these, acacia yielded good growths. The best combination proved to be a saturated solution of powdered acacia in nutrient agar, having a slightly acid reaction. The medium quickly becomes wrinkled, and is of no value aside from indicating a fondness of the gonococcus for the acacia enzyme. Certain animal substances containing enzymes were similarly employed, viz., pepsin, pancreatin, and ptyalin, pepsin and pancreatin yielding important and surprising results. The preparations of pancreatin used were the ordinary pancreatic substance (insoluble) and a purified pancreatin rendered soluble by acetone fractioning. Both of these preparations in one to two per cent strengths in slightly alkaline, neutral, and slightly acid nutrient agar influenced the growth of gonococci in a marked degree. In no medium was the growth so luxuriant and rapid as in the neutral pancreatic media, but the organisms at the same time appeared to undergo an extraordinarily swift autolysis. They appeared well-nigh structureless and stained very poorly with the strongest acid stains. Growth in the acid medium was somewhat slower, but still copious, and the organisms were better preserved. The growth is very short-lived. Daily transplantations are necessary, and even then strains die suddenly. Growth is not invariable. Occasionally inoculations will not "take" at all, but the thick, opaque, yellowish-white, smeary, tenacious mass quickly covering the entire surface of the medium is quite characteristic.

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[1] *Jour. Med. Res.*, 1909, 20, p. 365.

Soluble pepsin in the same proportions and reactions also yielded abundant growths, but with a slightly acid reaction, quite rapid and showing much better viability and staining reaction, and less autolysis. Like pancreatin, it yielded irregular results, but one is inclined to the opinion that all irregularities in "takes" might have been due to variations in technic rather than to special properties of media or strains, since the same irregularities are to be observed with other media routinely employed. Not enough work has been done with these enzymes to warrant any conclusion, but the results indicate a predilection of the gonococcus for certain enzymes and an antipathy for others. Sterile preparations of pepsin and pancreatin are difficult to obtain. Dry heat just short of 100° C. changes the color of the products, although it is said not to destroy the activity of the ferments. Boiling destroys the enzyme. Good results were obtained by heating solutions of pepsin and pancreatin to 60° C. for one hour on three successive days. These substances are distinctly acid in reaction and require the addition of alkali, preferably NaOH solution, before being added to culture media. The addition of alkali also renders the substances more readily soluble. Further mention of the enzyme relationships will be made later in this paper and, it is hoped, more fully in a subsequent communication.

Stab cultures and anaerobic culture media in general are not favorable to growth of gonococci, although unquestionably some growth does occur, provided the media contain the ascitic fluid or other favorable substance. This we should expect to be the case from our knowledge of the pathology of gonorrheal infections. The organisms survive for considerable periods of time without air. Voluminous cultures upon ascitic agar placed in a vacuum chamber over calcium chlorid and kept at 20° C. for two weeks survived and subcultures were successful.

MORPHOLOGY.

The morphology of the gonococcus is so variable as to have given rise to considerable discussion. My experience has been that by using many strains and by varying the media on which they are grown, one can observe all the types of the entire coccus family. By undoubted processes of reversion and variation it is possible, and common, to observe staphylococcus-like clumping of single forms, short chain formation as with the streptococci, lanceolate forms indistinguishable from pneumococci in shape, diplococcal forms resembling the catarrhalis groups, all in unquestionably pure cultures of gonococci. All return promptly to strain type on appropriate media. For instance, the strains grown in fluid media show the most lanceolate and chain forms; those grown on an over-dry medium contain the larger number of clumps, while the cocci bred on moist ascitic agar of definite salt content and uniform reaction yield the fewest variants. From the examination of many hundreds of cultures and thousands of preparations, my opinion concurs with the view that the gonococcus is classed properly as a micrococcus. A majority of the forms are single, irregularly round and discrete. Diplococcal forms, biscuit or coffee-bean in shape, follow numerically and appear to denote active proliferation. Still a larger number of cocci in all cultures show variation and irregularity. Variations in size are common in different media. By varying the salt content of ascitic agar, for instance, one is able to breed cocci of all sizes from the small staphylococcus size to those having a diameter equal to half that of a red blood-cell. The largest cocci were produced on salt-free ascitic agar, the smallest and most stable organisms on human blood agar; among the larger cocci were many ring forms with the staining portion at the periphery and the centers shadowy.

The gonococcus is difficult to stain well. After having used a multitude of ordinary and special stains, it was found that the sharpest contours and the most brilliant staining are obtained with carbol fuchsin, one part of the ordinary tubercle carbol fuchsin and three of distilled water. Strangely enough, the methylene blue stains are very unsatisfactory for work with gonococcus cultures. The organisms do not take the stain deeply and the contours are not clear cut or sharply defined. The variations in character of different stains will embrace all variants, even for complement-fixation work.[1] My own observation would designate six selected strains as sufficient to cover all characters. The usual variations in appearance, consistence, colony forms, etc., are too well known to require further comment. The best descriptions and illustrations of cultures and organisms are by Martin.[2]

AUTOLYSIS.

It has been a matter of common observation for some time that gonococci suspended in water and in salt solution disintegrate with more or less rapidity, so that eventually no organisms whatever can be distinguished. To this peculiarity has been given the name autolysis, which is particularly apt since the property of solution or disintegration seems to be one inherent with the gonococcus. The autolysis is quite comparable to that which Rosenow and others have demonstrated in the pneumococcus. That the substance which causes the solution of the bodies of the cocci is itself a product of their life and activity is shown by the fact that autolysis occurs when all other possible causes have been removed. Gonococci which have been washed three times in distilled water, once in 0.125 NaOH solution, once in 1:5000 HCl solution, will, when again suspended in salt solution or distilled water, disintegrate with the same rapidity as unwashed cocci freshly removed from the culture medium. No culture or colony of gonococci is so young but that autolysis has already begun. Observation has shown that the life history of the individual gonococcus is, in its own sphere, comparable to the ephemeridæ. Within an amazingly brief period the sequence of coccus, diplococcus, and disintegration occurs. As each organism dies it contributes a heritage which is cumulative and hastens the death of its fellows, until within the space of a few days a culture of untold millions of the cocci has perished, destroyed by the combined activities of its individuals. In transplanting cultures over 48 hours old larger quantities must be transferred in order that the few live cocci therein may be included.

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[1] Torrey, Jour. Med. Res., 1907, 16, p. 329, and 1908, 19, p. 471; Schwartz and McNeil, Am. Jour. Med. Sc., 1911, 141, p. 693.

[2] Martin, Jour. Path. and Bact., 1911, 15, p. 76.

Agents which check autolysis.—McClintock and Clark[1] have shown that a temperature of 70° C. for one hour effectually checks disintegration of suspensions of gonococci in salt solution. Temperatures less than 70° C. with or without the addition of tricresol 0.2 to 0.4 per cent do not avail.

Hydrochloric acid solution in distilled water in strength as low as 1:5000-1:10000 is the best preservative of gonococci. Suspensions of gonococci in the medium remain unchanged for months, and serve as the most stable vaccine that has so far been prepared. Tricresol may be added if desired, preferably a strength of 0.2 per cent. The gonococci are quickly killed in the solution. Organisms preserved for months in this manner resume autolysis upon washing and suspending in salt solution. Hydrochloric acid solution produces a strong agglutination of the gonococci, a fact which necessitates breaking up of the clump by shaking with glass beads, in the preparation of vaccine.

Lactic, acetic, nitric, benzoic, and oxalic acids in weak solutions in distilled water are also preservatives of the bodies of gonococci. It appears that almost any weak acid tends to overcome autolysis.

Glycerin solution five per cent in distilled water is a good preservative for short periods of time. Pepsin, 0.5 neutral, or slightly acid solution in distilled water is almost as good a preservative as dilute hydrochloric acid, but a slight yellowish-brown discoloration occurs on standing for a considerable time. Heavier pepsin percentages cause digestion.

Alcohol solutions as high as 10 per cent, weak solution of formalin, chloroform water, all sufficiently strong to prevent bacterial growth, appear to have little or no influence upon autolysis, providing the reaction of the solution is neutral or slightly alkaline. In conducting experiments on digestion one not infrequently adds some substance like chloroform, which prevents bacterial growth, but in no way hinders the activity of the digestive enzyme, and it is noteworthy that the same substances do not prevent the autolysis of gonococci.

Agents which favor autolysis.—Salt solution 0.8 to 0.9 per cent and distilled water are very favorable agents. If to either there be added *n*/10 NaOH solution or other alkali sufficient to produce slight alkalinity, it is observed that autolysis is materially hastened. Normal blood alkalinity (0.125 approximately) is a very favorable degree. It was also observed that addition of small quantities of those proteolytic enzymes which operate in an alkaline medium hastens lysis of the gonococci. In particular, it was found that the most rapid lysis of all occurred when the organisms were suspended in a slightly alkaline solution of pancreatin and preserved at 37° C. Beautiful illustrations of the disintegration may be observed in hanging-drop preparations where the process in a single field may be watched, and in stained preparations made from hour to hour.

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[1] *Jour. Infect. Dis.*, 1909, 6, p. 217.

Digestive powers of autolysates and dried gonococci.—Autolysates of gonococci were prepared by suspending 24-hour cultures in 0.9 per cent salt solution, allowing the suspension to stand at room temperature for three days, shaking with quartz sand for three hours, after which the débris was removed by passing the fluid through a Berkefeld filter. The filtrate had marked toxic effects on rabbits and guinea-pigs, three cubic centimeters being sufficient to kill a rabbit of 1500 gms. in half an hour.

Gonococci were dried by suspending larger cultures in as small a quantity of distilled water as practicable and evaporating the fluid in a vacuum chamber. The dried substance is light brown in color. Cultures from 12 whiskey-flask surfaces yield a little over one gram of dried substance. These are also highly toxic.

With these substances the following digestive test was made:

Controls: 1. Pancreatin solution 0.020; salt solution 4 c.c.
2. Gonococcus autolysate 4 c.c.
3. Dried gonococci 0.020; salt solution 4 c.c.
4. Fibrin 0.020; salt solution 4 c.c.

Determinants: 1. Pancreatin 0.020; salt solution 4 c.c; fibrin 0.020, alkaline to litmus.
2. Gonococcus autolysate 4 c.c.; fibrin 0.020, alkaline to litmus.
3. Gonococcus autolysate 4 c.c.; fibrin 0.020, acid to litmus.
4. Dried gonococci 0.020; salt solution 4 c.c.; fibrin 0.020, acid to litmus.
5. Dried gonococci 0.020; salt solution 4 c.c.; fibrin 0.020, alkaline to litmus.

To each tube was added a few drops of chloroform and all were placed in the incubator at 37° C. for eight hours.

Result: Controls 1, 2, 3, and 4—Negative Biuret.
Determinants 1 = + Biuret
2 = ++ Biuret
3 = + Biuret
4 = ++ Biuret
5 = + Biuret

It seems reasonable to assume that the gonococcus produces a proteolytic enzyme, similar to trypsin, which has a temperature range of viability between 0° C. and 70° C. It will be shown later that the physiological effects of the enzyme on laboratory animals are very strikingly similar if not identical with those of trypsin. It appears that the enzyme is capable of slight activity in an acid medium, although a slight HCl acidity is capable of preventing its formation. It is a question whether this substance or enzyme can be classed with the toxins in the sense that we refer to the soluble toxin of the diphtheria bacillus and the endotoxin of the typhoid bacillus. The so called toxins of the gonococcus, the meningococcus, and the pneumococcus, and perhaps also the streptococcus, appear to form a group by themselves. Certainly those of the gonococcus and the pneumococcus present strikingly similar features.

AGGLUTINATION.

Tests of agglutination of gonococci with antisera have been disappointing in my hands. The claims of many to have produced sera which will agglutinate in dilutions of 1:50,000 to 1:200,000 I have been unable to substantiate. The most powerful serum in my series was that of a rabbit which had received three inoculations only, as follows:

February 5, 1912: Intravenously, the autolysate of one ascitic agar tube of gonococci kept for 10 days at 37° C. in two cubic centimeters of rabbit serum.

February 20, 1912: Intraperitoneally, one large loop of 24-hour ascitic agar culture of gonococci in two cubic centimeters of sterile salt solution.

February 27, 1912: Intraperitoneally, one ascitic agar tube culture suspended in two cubic centimeters sterile salt solution.

The animal was bled for serum five hours after the last inoculation.

April 20, 1912: Agglutination test.

Standard suspension of gonococci in distilled water.

Serum dilutions: 1:1 = + at once
1:1000 = + at once
1:50000 = + in one hour.

By way of comparison, a control agglutination test was made with an antigonococcus serum (017326 K. B1095) on the same day. The results were as follows:

Dilution 1:10 = negative in two hours; partial in 18 hours.
Dilution 1:40 = negative in two hours; slight in 18 hours.

Antimeningococcus serum (018781) gave a positive agglutination of gonococci in the dilution of 1:10 in two hours.

Antidiphtheric serum, normal horse serum, and certain guinea-pig sera give positive results in eight hours' time, but almost any serum will do the same, whereas those sera produced by long-continued immunization of rabbits with large quantities of gonococci do not show that degree of agglutinative power one would expect. For instance, one rabbit received in all 16 inoculations, the first 12 intravenously at intervals of five days, the last four intraperitoneally at 10-day intervals. This serum was positive in a dilution of 1:10 in three hours and negative in a dilution of 1:40.

The substance which invariably produces the most marked agglutination of gonococci is HCl in dilution of 1:2500 and as high as 1:10000 in distilled water. Salt solution with HCl in the latter dilution is not favorable and, indeed, permits some autolysis.

Gonococcidal substances.—All ordinary germicidal substances are fatal to the gonococcus. Indeed, distilled water is destructive and salt solution even more markedly so. On the most favorable culture medium the life of the organism is very short at best. It was impossible to determine whether the silver salts, those of mercury, the acids, the alkalies, formol, the essential oils, the halogens, all in the strength usually employed clinically, were fatal, one sooner than the other, since all were quickly fatal. The experiments of Wildbolz,[1] Paldrock, and others upon the viability of the gonococcus in germicidal solutions are valuable and of great interest, but the brief periods of time, even to the fractions of minutes mentioned by some, reveal what a painstaking procedure is demanded.

ANIMAL EXPERIMENTS.

All efforts to produce a specific inflammation of the eyes or genitalia of animals were futile, as were the efforts to produce abscess, by inoculating animals with fresh cultures of gonococci.

The mechanism of defense of the mucous surfaces of all animals is much the same. The eye of the rabbit furnishes a good example. Five minutes after the conjunctival sac has been smeared with freshly isolated pure 24-hour cultures of gonococci there appears at the inner canthus a globule of grayish exudate which is seen in stained preparations to consist of mucus and immense numbers of epithelial cells, clinging to which are larger clumps of gonococci which stain poorly and appear to be undergoing rapid lysis. There are but few polymorphonuclear leukocytes. After 15 minutes the picture is much the same except that the cocci are much diminished in number. In 30 minutes the exudate has become more watery and consists of epithelial cells and leukocytes, but evidence of phagocytosis is wanting. In an hour, all cocci have disappeared and the eye appears normal. At no time does the eye show inflammatory reaction. All efforts to hold the culture in place in the conjunctival sac by mechanical means failed. The submucous injections of cultures were also negative.

The few positive results obtained in animals will be briefly outlined.

Monkeys (M. rhesus) small; 7 pounds weight.—No. 1. Hypodermic injection of morphin sulphate 2 grains; intradural injection of one half tube of ascitic agar 24 hour culture suspended in 2 c.c. normal monkey serum. Elevation of temperature (104.5° F.), coma, convulsions, and death in nine hours. Autopsy showed local fibrin-like exudate at site of inoculation and reddening of the meninges. Cultures negative. Exudate showed a few poorly stained shadowy cocci, leukocytes, and débris; no organisms.

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[1] Arch. f. *Dermat. u. Syph.*, 1903, 61, p. 151.

In the left conjunctival sac of the same monkey there was placed one-half tube of ascitic agar tube culture 24 hours old on the end of a spud synchronously with the intradural injection. Postmortem appearances: right eye normal; left eye protruded, lids everted and swollen, conjunctiva chemotic. Dirty gray exudate in moderate amount containing leukocytes, epithelial cells, and débris. Smears and cultures negative for gonococci. Sections of lower lid and conjunctiva showed inflammation and edema but no organisms.

No. 2. Morphin sulphate 2 grains. Intravenous injection of one-half tube of ascitic agar tube culture of gonococci, 24 hours old, in two cubic centimeters salt solution. Elevation of temperature (106° F.), coma, death in 12 hours. Endocardium over chambers and valves appeared lusterless and "smoked." Smears from liver and spleen showed poorly stained, irregular, shadowy cocci. Phagocytosis absent. Cultures from heart's blood on ascitic and blood agar positive.

No. 3. (Control)—Morphin alone. Recovered.

No. 4. (Control)—Intravenously one-half ascitic agar tube culture in two cubic centimeters salt solution. Temperature maximum 104° F. Recovered.

No. 5. (Control)—Intraperitoneally, one-half ascitic agar tube culture in two cubic centimeters salt solution. Recovered.

Rabbits.—It was demonstrated that phagocytosis plays small part in the immunity of these animals, as well as in other laboratory animals. This statement is based on a large number of examinations of blood and peritoneal exudates in inoculated animals, and in many phagocytic experiments *in vitro* with the blood of animals.

Rabbits react to both intravenous and intraperitoneal injections of gonococci, with prompt rise of temperature, which quickly returns to normal.

Guinea-pigs.—Guinea-pigs of all laboratory animals manifest the nearest approach to a true septicemia following inoculations with cultures of gonococci. The response is prompt and definite and in every instance where a fatal dose was given, gonococci were recovered in cultures from the heart's blood and the organisms were demonstrable in smears from the liver, spleen, and lungs. Phagocytosis is not a conspicuous feature, although it appears to be somewhat more common in this species than in others. The initial fatal dose of most strains is the 24-hour growth of one ascitic or blood agar tube. This was suspended in two cubic centimeters of sterile salt solution and injected into the peritoneal cavity. The animal is at once ill, and the temperature begins to fall and continues to fall steadily until the animal dies in from five to twelve hours. Animals surviving longer than 12 hours usually recover. The peritoneal exudate after one hour is serous and tinged with blood. It contains numerous red cells, innumerable well staining gonococci, and only few leukocytes of any kind. The few polymorphonuclear cells observed seldom contain cocci. After three or four hours, and after death, the fluid exudate, about 3 to 5 c.c. in amount, is grayish and contains flakes of lymph. The peritoneum is flecked with lymph, particularly about the liver and over the intestine. At this time the exudate contains greater abundance of leukocytes, but still the polymorphonuclear cells are few and phagocytosis is not conspicuous. Those cocci which live within the cells are ragged, misshapen, and do not take the stain well. Gonococci are still numerous but are rapidly diminishing in numbers and losing their outlines. The liver and spleen are usually dark red, enlarged, and congested. Cocci are always demonstrable in smears from the pulp of these organs, being for the most part free, while a few poorly stained organisms are seen within the leukocytes. Cultures from the heart's blood are almost invariably positive, and from this fact it was learned that the quickest and surest method of purifying contaminated strains of gonococci is by injecting the culture in large quantities into guinea-pigs intraperitoneally, and inoculating the heart's blood on blood agar five hours later, after chloroform death.

The strain of gonococci resulting from such cultures was used over and over again, passing it from tube to pig and pig to tube regularly every 24 hours for 30 days. The strain then appeared to have lost some of its autolytic tendency; it took the ordinary stain better, became more hardy and adapted itself readily to ordinary nutrient agar (reaction +0.7), on which medium it survived eight days at 37° C. and was carried on through 22 subcultures. The impression was gained that the gonococcus is capable of modification by environment into an organism of fair stability, in spite of the observation that continued operation of the same strain through guinea-pigs did not appear to lessen the quantity required for a lethal dose.

STUDIES ON THE VIRUS OF HOG CHOLERA*†

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INTRODUCTION.

The terms "filterable virus" and "ultramicroscopic organisms" are frequently used in the descriptions of some 30 important infectious diseases of man and animals. These terms constitute a convenient form of expression, as in certain cases it would otherwise be necessary to state "specific cause unknown."

Among the important diseases which fall into this class, as enumerated and briefly described by Wolbach,[1] are yellow fever, molluscum contagiosum, dengue fever, verruca vulgaris, trachoma, sand-fly or three-day fever, acute anterior poliomyelitis, measles, typhus fever, and scarlet fever, which affect man only; rabies, foot and mouth disease, variola, and vaccinia affect both man and animals; whereas hog cholera, fowl pest, cattle plague, sheep pox, African horse sickness, swamp fever of horses and Rous's chicken sarcoma are among those affecting animals only. Canine distemper, which Wolbach mentions as being produced by a filterable virus, has been demonstrated by Ferry[2] to be due to a bacillus termed by him *B. bronchisepticus.*

Since the recognition of the filterability of hog cholera virus by De Schweinitz and Dorset, 1903-1905, very little progress has been made in elucidating the nature of the specific etiological factor involved.

Dinwiddie,[3] from a series of carefully planned centrifugation experiments, concludes that the infectious agent involved in hog cholera is more or less closely associated with the red blood corpuscles. Aside from the report of Dinwiddie, the knowledge

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†Read before the Society of American Bacteriologists, January 2, 1913.

[1] *Boston Med. and Surg. Jour.*, 1912, 167, p. 419.

[2] *Jour. Infect. Dis.*, 1911, 8, p. 399.

[3] *Bull.*, Arkansas Exp. Sta., 1912, No. 111.

accumulated in regard to this subject is relatively meager and scattered.

The filterability of a given virus does not preclude the possibility of the presence in that virus of microorganisms relatively large at certain periods, according to the stage of development in their life cycle. Borrel[1] demonstrated a protozoan, *Micromonas mesnili*, which, during certain stages of its development, would pass through the Berkefeld and Chamberland filters.

Loeffler also has shown that a flagellate belonging to the genus Bodo would pass through Berkefeld filters, which were capable of preventing the passage of *B. prodigiosus*, even after an hour's filtration. Thus, filterability cannot be taken as an indication of the size of a given organism, because the passage of organisms through filters depends also on their plasticity and their ability of accommodation to the pores of the filter. In this, the smaller forms of motile parasites differ from bacteria of relatively the same dimensions. It therefore seems possible that some of the filterable viruses causing disease may have a stage in the cycle of their development which is visible under the microscope.

Betegh[2] of Hungary has succeeded in retaining hog cholera virus by means of the Bechhold ultra-filter. His experiments consisted in the filtration of two different strains of virus and the injection of each of the filtrates into three susceptible pigs. The six pigs remained well and the conclusion was that the virus had remained in the ultra-filter.

In 1910,[3] a comparative histological study of the blood of normal hogs and cholera-infected animals was conducted. In this work, the blood of hogs suffering from cholera, in comparison with normal blood, was studied in a routine way on the hemocytometer and with the ordinary blood stains, but at the same time much care was exercised in attempting to find any differences which might indicate the probable nature of the causative factor. Aside from the data obtained relative to the ordinary blood examinations, nothing of note was observed in the specimens of hog cholera blood.

A few months ago a comparative study of normal hog blood and blood from animals suffering from hog cholera was undertaken, in order to determine whether any differences could be detected by means of the dark field method of illumination. A preliminary report[1] of this work has appeared.

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[1] *Ann. de l'Inst. Pasteur*, 1903, 17, p. 81.

[2] *Berl. tierärztl. Wchnschr.*, 1912, 28, p. 969.

[3] King and Wilson, *Bull.* Kansas Exp. Sta., 1910, No. 171.

The fresh blood of normal and diseased hogs was collected aseptically in sterile sodium citrate solution and kept in the incubator until the examination was completed. The blood was obtained from the caudal artery after a small portion had been clipped off with a sterile instrument. In this way bacterial contamination was reduced to the minimum.

In the blood of both normal hogs and animals suffering from hog cholera, as in the blood of any animal, many peculiar structures are observable. The blood of normal hogs thus examined on the dark field may show, in addition to the normal structures, a few granular bodies, sometimes a few bacterial cells from possible extraneous contamination and filaments. The latter may assume the form of "dumb bells," "chains," "droplets" or flexible filaments, which by an untrained observer might easily be mistaken for spirochetes. An excellent description of these bodies as well as others found in the blood of normal animals is given by Balfour.[2]

Betegh, in examining the hog lymph and serum respectively, from two animals dead from hog cholera, evidently has mistaken some of these filaments, described by Balfour, for bodies of some possible significance. In his very recent article on ultrafiltration experiments, Betegh[3] describes, in a rather confused way, his limited dark field finding as follows:

> "Series I. January 16, 1912. Animal infected and died with hog cholera under natural conditions. Pathological changes: Typical stratification (button ulcers) in cecum and colon; edematous infiltration along the arteria coronaria cordis; infiltration in the right apex of lung; disseminated infiltrated areas in the right lung; parenchymatous degeneration of the kidneys; hemorrhagic inflammation of the lymph glands. Diagnosis: Hog cholera. Microscopic findings: Scattered bipolar and other (colon) bacilli in the organs. In the lung lymph beside these, small slightly stained bodies. In the dark field examination, actively motile, massed bodies from 0.3 to 0.5 microns in size are visible, which are

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[1] King and Baeslack, *Jour. Infect. Dis.*, 1913, 12, p. 39.

[2] *Fourth Report,* Wellcome Tropical Research Laboratories, 1911, p. 109.

[3] *Berl. thierärztl. Wchnschr.*, 1912, 28, p. 972.

strongly light-refractive. Many appear to have a small apophysis. Very often dumb-bell like forms are seen, and further, spirochete-like microorganisms, which have at each end a light-refracting round knob. Cultural: On alkaline agar these have grown at 37° after 24 hours, many coli, bipolar, and bacterial colonies similar to the *B. suipestifer*."

"Series II. April 16, 1912. Swine sickened and died under natural conditions. In colon a typical button ulcer of the size of a small coin. Several lentil-sized ulcers at the point of anastomosis of the small intestine into the colon. The mesenteric lymph glands swollen and hemorrhagically inflamed. The apex of both lungs infiltrated, atelectasis. The pleura was weak and covered with pseudo-membranes. Surface of incision of the lungs marbled; beside yellow necrotic groups, different stages of hepatization of partial pneumonia were visible. Diagnosis: Hog cholera. Microscopically with diluted carbol fuchsin solution, numerous, short bacteria, rounded off at both ends, occasionally typically bipolar colored, were visible. With the Giemsa staining, in part, the same form. Numerous 0.3 to 0.5 micron sized, ovoid or round forms were seen, which seemed to arise from a chromatin substance. In colored streak preparation from the button ulcers, intra- and extra-cellular typical spirochetes were seen. Similar bodies were abundantly visible with the dark field in the serum. They were actively motile. Between small dumb-bell shaped forms were seen also spirochete forms."

From the above description it is quite evident that Betegh based his observations on the fallacies to which Balfour has so aptly called attention. In the blood of different species of normal animals, filaments may be observed which at times closely simulate spirochetes. Most of these "pseudo-spirochetes," as seen in blood on the dark field, present a knobbed appearance at each pole. After one has become accustomed to the appearance of these filaments, one can readily distinguish the lack of motility, in spite of a flexuous distortion, which is assumed as the filaments are carried by a slight current in the fluid of the preparation. These filaments are also lacking in refractibility and are relatively slender in comparison to the spirochetes recognized in this work.

Bacilli are easily recognized in the dark field by the complete rigidity of the cells and, when flagella are present, by the characteristic tumbling motion.

Results from the dark field examinations of specimens of blood from some 50 normal hogs indicate that, as a rule, the blood is relatively free from granules. Under ordinary conditions, and with few exceptions, the blood of normal hogs examined has been designated in our notes as "clean." There have been some few instances in which the blood of hogs unexposed to cholera, and in apparently normal condition, have shown the presence of numerous granules. The presence of these granules in normal blood in the majority of cases has been traced to ruptured leukocytes.

In the specimen of blood from all infected hogs, which have been observed by means of the dark field, a relatively large spirochete has been found. It averages from five to seven microns in length and one micron in width. The body of the organism is flexible and round at its ends. It presents no knobbed appearance at its poles. Actively motile, it revolves about its longitudinal axis. Its motility is undulating in character and its spirals are fixed. A few of these organisms have been observed dividing longitudinally. In one permanent microscopical mount, prepared by india ink fixation, one of these organisms apparently shows a polar flagellum. On the dark field this spirochete is readily distinguished from bacteria on account of its lack of rigidity and its characteristic motility, and from "blood filaments" by its greater refractive properties and characteristic morphology.

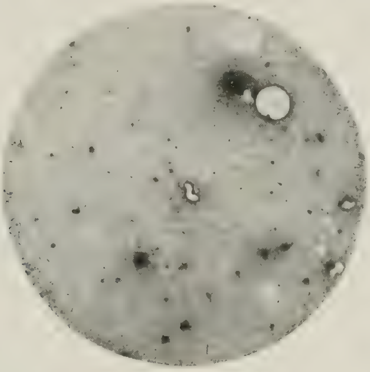

FIG. 1.—Microphotograph of spirochete in blood of Hog 504. India ink preparation.

This spirochete has not been found in large numbers, in any of the blood preparations. However, in nearly every specimen examined, more than one has been observed and in many cases five or six have been found with little difficulty. As a rule the organisms have been found to be more numerous at the height of the disease. The specimens of blood examined have been diluted in the proportion of about one to ten or fifteen with sterile sodium citrate solution, which factor should be considered in contemplating the number observed in a given positive specimen. Moreover, it is suggested that this organism, when observed as a spirochete form, constitutes only one stage of its development.

Certain types of granules appear to be characteristic of blood from cholera hogs. It usually contains many granules, some very fine yet more distinct than blood dust, some larger still, and some very distinct, highly refractive bodies. In many specimens of cholera blood were observed innumerable small granules, which were much more definite and distinct in outline than blood dust, and easily differentiated from the whitish, partially refractive granules from ruptured leukocytes and from the more highly refractive and larger bodies composed of débris, bacteria, and filaments. In this work the presence of these granules appeared to be so characteristic of blood from cholera hogs, that it became an invaluable aid in finding the spirochetes. While these particular bodies may be disintegrated blood elements resulting from disease processes, yet it may properly be suggested that some of them may represent certain stages in the life cycle of the spirochete which has been observed.

The majority of the hogs used in these experiments were inoculated with virus which was diluted 1 to 1 with sterile physiologic salt solution and filtered through Berkefeld filters. In the filtrates only a few granules were visible when studied on the dark field.

In this connection it should be mentioned that the majority of the dark field examinations which are included in this work have been checked by each of us. In addition to the above means of controlling the results, specimens of blood were at times collected by an assistant who designated the specimens by symbols. As the daily examination often included specimens from both normal and diseased animals, the results were put to a practical test, and in no instance did the results from the dark field examination deviate from the clinical conditions of the animals whose blood was examined. In some instances, as will be shown by the following detailed results, the presence of hog cholera infections was practically detected by the dark field examination before it was known that the animals showed any symptoms of the disease.

It is interesting to note that in 1894 Dr. Theobald Smith[1] submitted the following brief report on "Coarse and Fine Spirilla in the Intestine of a Hog":

> "The recently published articles on fine spirilla in the excrement of cholera subjects prompt me to communicate an observation from animal pathology. Early in 1889 I found non-liquefying comma bacilli in small abscesses of the large intestine, in a hog which I have briefly described in this journal. In streak preparations of the same, stained with alkaline methylene blue, I found, besides large quantities of vibrios, also a great many fine spirilla, of two to three wave-lengths. The wave-lengths of fixed form are about two microns. A preparation, now more than five years old, still shows the tiny spirilla very distinctly. At that time they never appeared again in cultures. Further investigations concerning the presence of this organism in other animals have not been made."

Betegh, in a lecture and demonstration given before the Veterinary Congress at Budapest in May, 1912, called attention to the spirochetes which were demonstrable in the ulcers of animals dead from hog cholera.

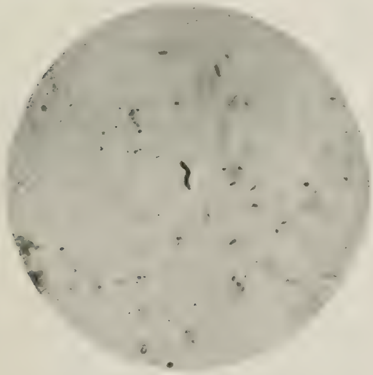

FIG. 2.—Microphotograph, spirochete stained by Giemsa method, from intestinal ulcer of Hog 556.

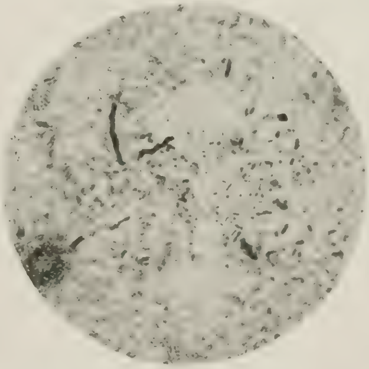

FIG. 3.—Microphotograph of spirochetes in impure culture, from cecal ulcer of Hog 551.

Little difficulty has been experienced in finding spirochetes in the intestinal ulcers of hogs dead from cholera. In the present work, the ulcers, as found in the cecum of cholera-infected hogs, have been obtained as free from contamination as possible, washed with sterile water, and intermittently scraped with sterile instruments. Portions of the scrapings from the diseased submucosa, after the above treatment, have then been examined on the dark field and in stained preparations, preferably by the Giemsa method. While it thus appears possible to demonstrate spirochetes in hog cholera ulcers with uniformity, yet a large variety of bacterial species of course constitute the flora. With present methods of study therefore, the recognition of these spirochetes in the intestinal lesions cannot be taken as of great significance. Whether or not some of these spirochetes in the intestinal lesions bear any relation to those present in the blood of cholera hogs is, of course, an open question. It is possible that the spirochetes found in the blood enter the lymph and blood stream at an early stage of the ulcer formation. This would offer a possible explanation of their comparatively small number as seen on any one blood mount.

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[1] *Centralbl. f. Bakt.*, 1894, 16, p. 324.

The details of the experiments may be better understood by a study of the following temperature charts and laboratory notes. The temperature charts include the time of inoculation, death or release of each animal, and other necessary data, including brief notations of clinical conditions and changes observed after death. The results of the dark field examinations are indicated by positive and negative signs placed above the temperature curves on the dates when the observations were made. In some instances the presence of granules or freedom of the blood from granules is indicated by abbreviations, gr. (granules) and c. (clean).

B.A.I. strain of virus.—The history of the strain of virus, designated in these notes as the "B.A.I. strain of virus," was supplied by Dr. Giltner of the Michigan Agricultural College as follows: "This is a representative of the original strain of Bureau of Animal Industry virus secured by Dr. Marshall at the conference at Ames in May, 1908. We have never kept any other strain of virus in the laboratory for any length of time. Practically all of our serum has been produced on the basis of this virus. It would be possible but very difficult to trace the passages through which this virus has gone since we first began to use it."

Beginning with Hog 446 the blood of practically all animals used in these experiments was carefully examined before inoculation in order to control the results.

Hog 446, inoculated with the B.A.I. strain of virus (Hog 444), manifested symptoms of the acute type of hog cholera after an incubation period of seven days. Three positive findings were made in the blood of this animal.

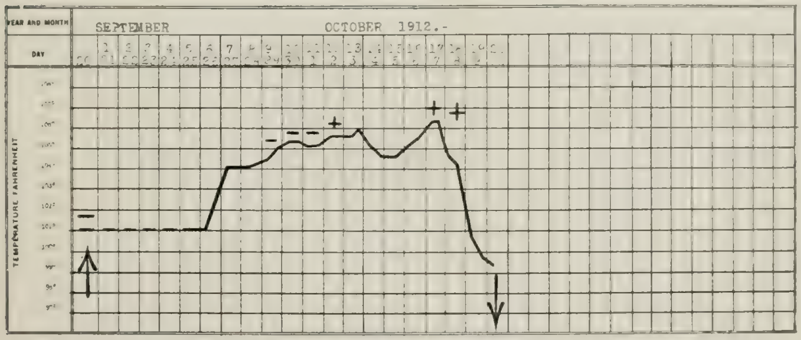
Results from the dark field examination of the blood of Hogs 453 and 454 afforded most interesting data. Both of these animals recovered after a mild course of the disease following a relatively long period of incubation. As the symptoms in both animals disappeared, dark field examinations failed to reveal the presence of the spirochete and the numerous characteristic granules. These hogs were kept under observation during a period of one month following recovery and were ultimately released as "immune."

Hog 455 was kept in a separate room in the experimental stable from October 26 until November 10 for the purpose of serving as a control on the methods used in isolating the experimental lots of hogs. During a part of this period Hog 455 suffered from a parasitic skin affection, but dark field examinations demonstrated a "clean" blood, free from granules and the spirochete. On November 10 the animal was exposed to the disease and developed symptoms in six days. His blood then showed positive findings and autopsy revealed lesions of cholera.

The charts of Hogs 506 and 507, inoculated with the B.A.I. strain of virus, need no further explanation.

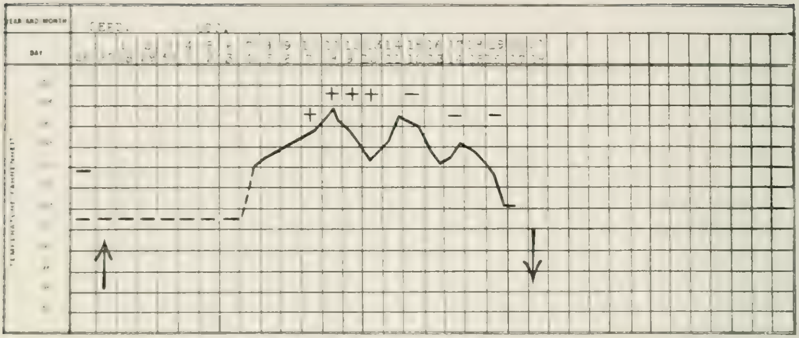
Michigan (Demerick) strain of virus.—On September 24 a farm at Roseville, Mich., was visited for the purpose of examining the herd of hogs. Several sick hogs of this herd, which had been isolated, manifested symptoms similar to those of hog cholera—malaise, anorexia, high fever, and diarrhea. A few purple areas were observed on the abdomen and ears.

Hog No. 446. B.A.I. Strain of Virus.



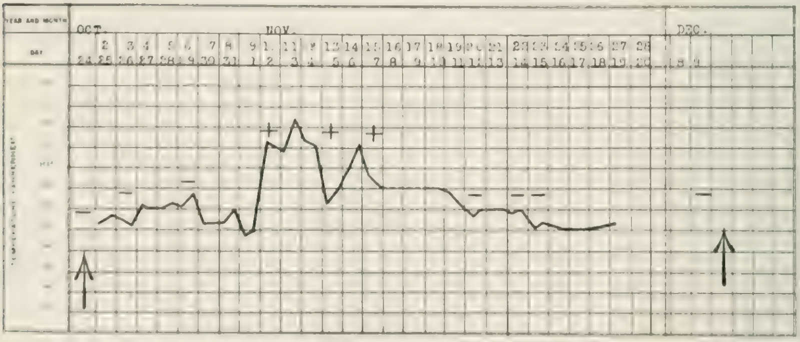
Remarks: September 20. Animal inoculated with 3 c.c. from 444 (B.A.I.).
September 27. Symptoms appeared.
October 10. Animal moribund, bled and examined.
Skin on ventral surface of body purple in color; hemorrhagic areas in subcutaneous and muscular tissues. Lymphatic glands enlarged and hemorrhagic. Kidneys show a few petechia, ulcers in cecum.

Hog No. 449. B.A.I. Strain of Virus.



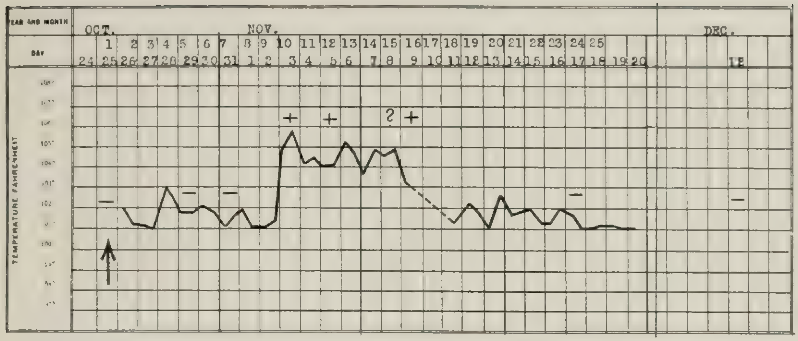
Remarks: September 27. Inoculated with 4 c.c. B.A.I. virus.
October 5. Symptoms appeared.
October 18. Animal found dead.
Purple hemorrhagic areas extending over greater portion of body externally and into subcutaneous tissue internally. Lymphatic glands enlarged and hemorrhagic. Ulcers in cecum. Kidneys petechiated. Areas of fatty degeneration in liver, right lung, upper lobe solidified.

Hog No. 453. B.A.I. Strain of Virus.



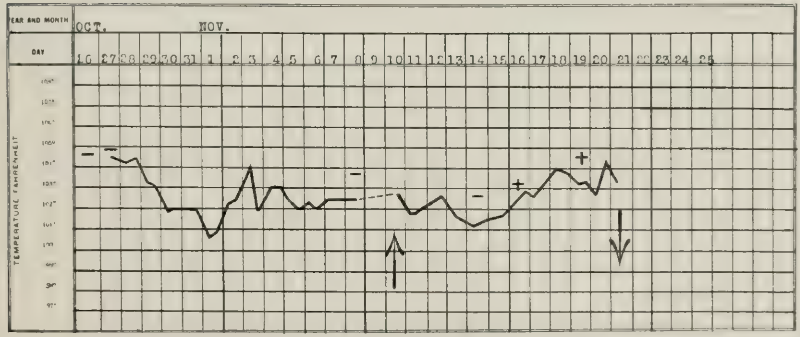
Remarks: October 24. Inoculated with 4 c.c. B.A.I. (filtered) virus, filtered through Berkefeld.
November 2. Symptoms appeared.
November 5. Blood positive.
November 19. Animal recovered.
December 15. Animal released, immune.

Hog No. 454. B.A.I. Strain of Virus.



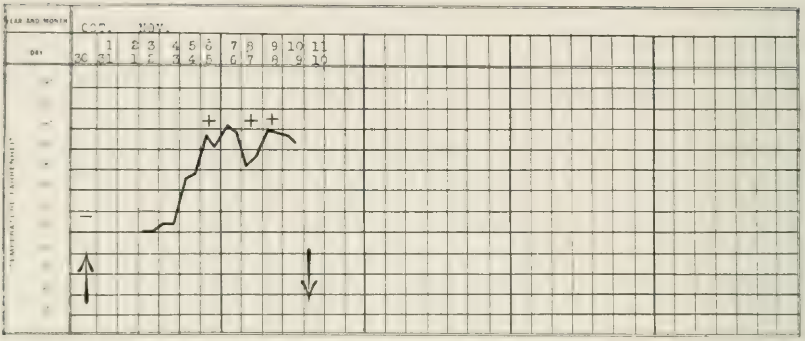
Remarks: October 25. Inoculated with 4 c.c. B.A.I. virus (unfiltered).
November 2. Symptoms appeared.
November 19. Animal recovered.
December 15. Animal released, immune.

Hog No. 455. B.A.I. or Michigan (Demerick) Strain of Virus.



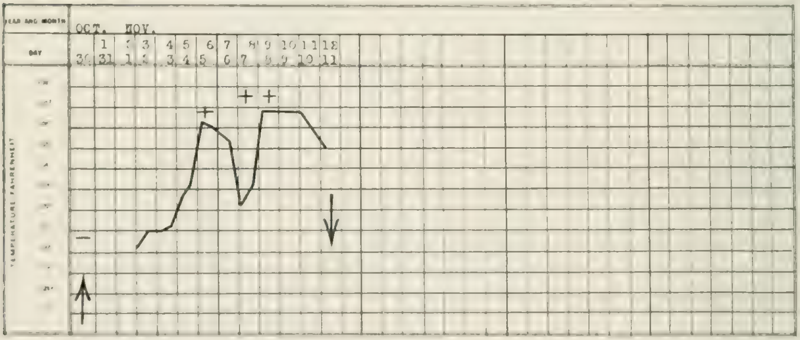
Remarks: *Control.* October 28. Appearance of "rash" on abdomen and flanks. Animal eats well and shows no symptoms of cholera. Diagnosed as parasitic skin affection.
November 10. Recovering hogs (not dipped) 446, 447, 448 placed in inclosure with 455.
November 16. Animal sick.
November 21. Pig moribund, bled and examined.
Typical lesions in cecum, lymphatic glands, and lungs.

Hog. No. 506. B.A.I. Strain of Virus.



Remarks:	October 30.	Inoculated with 4 c.c. B.A.I. virus.
	November 4.	Symptoms appeared.
	November 10.	Animal found dead and examined. Cecum, lymphatic glands, spleen, kidneys, and lungs show typical lesions of cholera.

Hog No. 507. B.A.I. Strain of Virus.



Remarks:	October 30.	Inoculated with 4 c.c. B.A.I. virus.
	November 4.	Symptoms appeared.
	November 11.	Animal found dead and examined. Lymphatic glands, cecum, lungs, kidneys, and spleen show typical lesions.

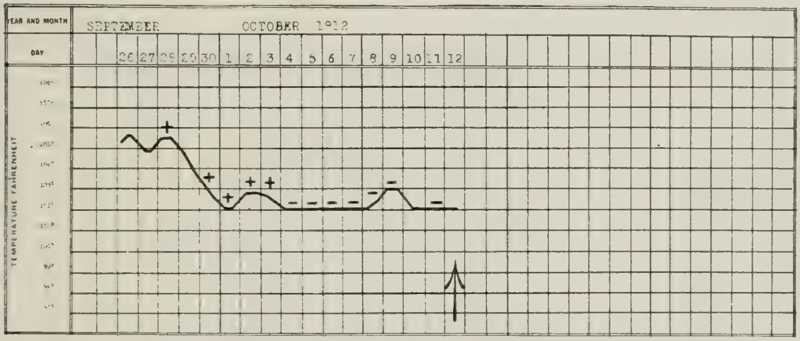
With the permission of the owner two of the animals were bled from the carotid artery and careful examinations made. Typical lesions of hog cholera were found in the large intestines, lymphatic glands, lungs, kidneys, and spleen, and a positive diagnosis of the disease in the acute form was made.

On September 26 two animals affected with hog cholera were brought to the laboratory. The blood of one of these hogs was examined immediately and the spirochetes were found with little difficulty. This animal was moribund, and was bled and examined on the same day. The other hog, No. 448, ultimately fully recovered from the disease. An examination of the temperature chart of hog 448 will show the logical results obtained from the study of his blood on the dark field.

Three hogs, Nos. 450, 451, and 452, were inoculated with the Michigan (Demerick) strain of virus. The results of the dark field blood examinations appear in the charts below and were clearly confirmatory of previous findings.

Michigan (Rochester I) strain of virus.—On November 9, eight apparently normal pigs were received from Rochester, Mich. No particular notice was taken of these animals as the blood of other hogs suffering from cholera was at the time under close observation. The eight susceptible pigs were received in an isolated pen, at some distance from the experimental stable, and cared for by an attendant who never entered the experimental stable. This was the routine procedure when normal pigs were received.

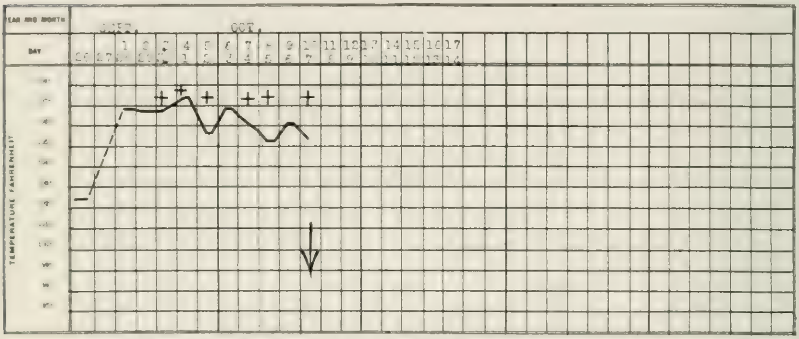
Hog No. 448. Michigan (Demerick) Strain of Virus.



Remarks: October 12. Animal fully recovered. Released as immune.

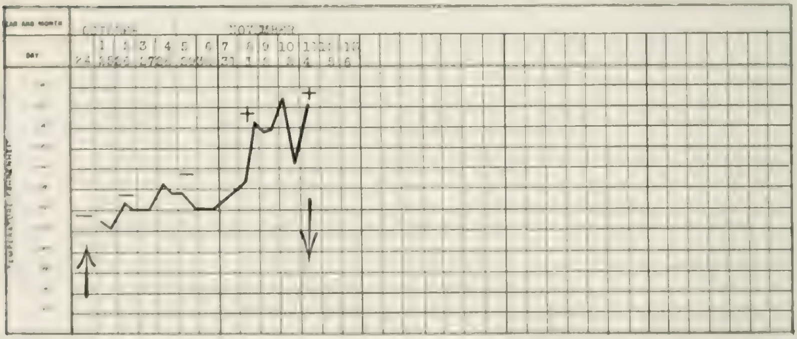
On the evening of November 12, Hog 503, one of the eight apparently normal pigs from Rochester, was removed to the experimental stable, inoculated with Michigan (Demerick) strain of virus and placed alone in a disinfected room. In this instance no dark field blood examination was made before inoculation, owing to the lack of time. It was planned that the examination of the blood, for the purpose of control, should be made the next morning following the inoculation. Likewise no temperature observation was made until the next morning. On November 13, the day following the inoculation of Hog 503, the dark field examination of the blood revealed the spirochete. The organism was again found on November 14. At this time the temperature of the animal was 104.2° F. and it showed symptoms of hog cholera. An inspection of the remaining hogs of this lot, which were still in the isolated pen used for receiving susceptible animals, showed that practically all were suffering from the disease. Among the apparent symptoms were diarrhea, anorexia and listlessness.

Hog No. 450. Michigan (Demerick) Strain of Virus.



Remarks: September 27. Animal inoculated with 4 c.c. Demerick virus.
October 5. Symptoms appeared.
October 14. Animal found dead and examined.
Large areas of congestion and hepatization in lower lobes of both lungs, kidneys contain a few petechiae. Spleen enlarged and gorged with blood. Mucosa of large intestine congested. Lymph glands enlarged and hemorrhagic.

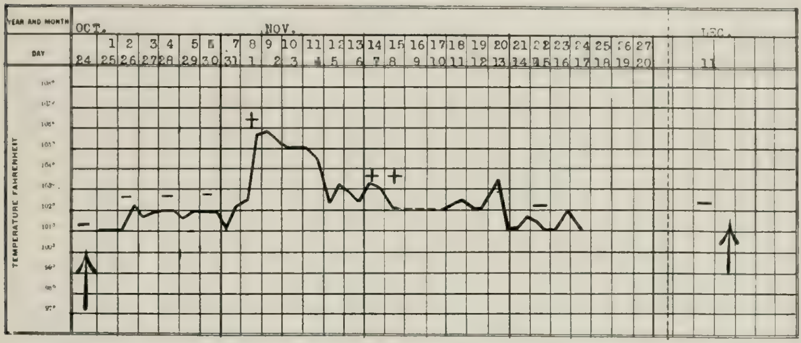
Hog No. 451. Michigan (Demerick) Strain of Virus.



Remarks: October 24. Demerick virus (filtered through Berkefeld).
November 1. Symptoms developed.
November 4. Animal found dead and examined.
Lesions in lymphatic glands, spleen, lungs, and cecum typical of cholera.

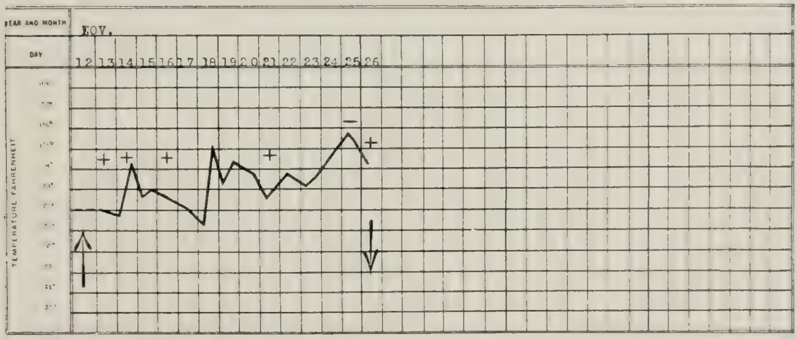
Careful inquiry was made as to the source of this lot of pigs and it was found that they were purchased from a stock buyer, who had kept them for 10 days previous to receipt in an enclosure which received all hogs purchased. Thus, it was clear that these pigs had been subjected to one of the most common methods of exposure to the disease, and that an incubation period of 10 days had elapsed before they were delivered at the laboratory.

Hog No. 452. Michigan (Demerick) Strain of Virus.



Remarks: October 24. Inoculated with 5 c.c. of Michigan (Demerick) virus (unfiltered).
November 1. Symptoms appeared.
November 17. Animal recovered.
December 15. Animal released, immune.

Hog No. 503. Michigan (Rochester) Strain of Virus.



Remarks: November 12. Inoculated with 4 c.c. virus from Hog 450 Michigan (Demerick) strain.
November 14. Found pig showing symptoms. One of Rochester hogs.
November 17. Typical symptoms.
November 20. Typical symptoms.
November 26. Animal moribund, bled and examined.
Lymphatic glands, lungs, kidneys, and heart muscle show typical lesions.

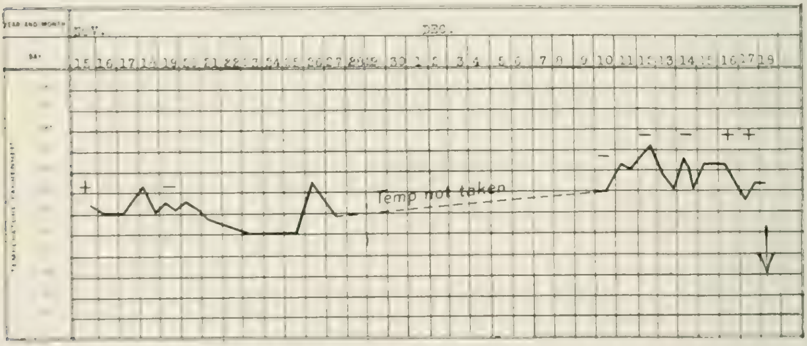
In this instance a diagnosis of hog cholera was practically made by means of the dark field.

The autopsy findings in Hog 503, which was bled two weeks after being taken under observation, could not consistently be used to verify the diagnosis of cholera in this lot of hogs, because of the fact that this animal had received an inoculation of Michigan (Demerick) strain of virus.

Hog 504 was killed and examined on November 16, after two positive dark field examinations were made. The lesions were not pronounced because of the fact that sufficient time had not elapsed for typical lesions of cholera to develop.

The disease in this lot was of the subacute or chronic type as is shown by a study of the following charts of Hogs 505, 509, 510, 511, and 513. The clinical conditions and autopsy findings left no room for doubt as to the nature of the disease.

Hog No. 505. Michigan (Rochester) Strain of Virus.



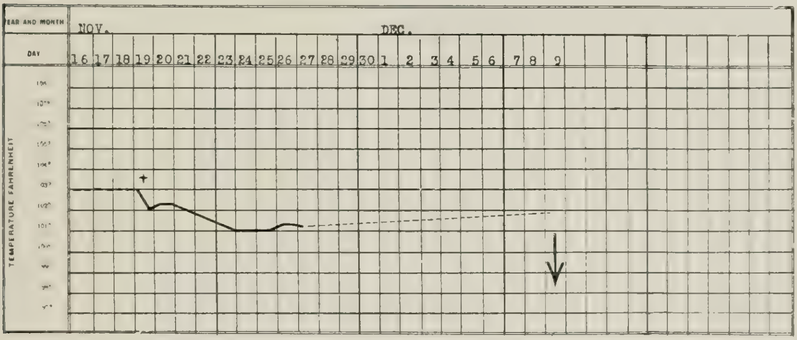
Remarks: November 15. One of Rochester pigs. Not inoculated.
November 20. Symptoms of chronic type.
December 10. Animal very weak and emaciated.
December 19. Hog died on bleeding table.
Lymphatic glands, heart muscle, lungs, kidneys, and cecum show typical lesions of cholera.

In further confirmation of the diagnosis of hog cholera in the above animals, when received at the laboratory, attention should be called to the chart of Hog 514. This animal remained in the isolated pen for susceptible hogs at the time the Rochester pigs were received. Hog 514 had been kept for a period of several weeks in the pen for "susceptibles" previous to this time and was in a healthy condition. The animal was not otherwise exposed to the disease, but promptly developed cholera about six days after the Rochester pigs were placed with it. The blood of Hog 514 showed the presence of the spirochete on three different examinations and autopsy revealed typical lesions of the disease.

Indiana strain of virus.—This virus was secured from Mr. Delplane and was originally supplied by Dr. R. A. Craig, who reported that this strain was secured by him in the field some time ago and did not represent the B.A.I. strain of virus.

Hog 501 was inoculated with 5 c.c. of Indiana I virus on November 7, after having made a dark field examination of the normal blood. On the seventh day after inoculation the spirochete was found in the blood of this animal, although symptoms, with the exception of a beginning rise of temperature, were not observed until the following day. Attention should be called to the fact that on the ninth day after inoculation no spirochetes could be found in the blood, but characteristic granules were present, while on the tenth day the spirochete was again found. This phenomenon, observed in other cases as well, suggests that the spirochete under observation represents one stage in the life cycle of the organisms, and that the granule formation is possibly an important factor in its development.

Hog No. 509. Michigan (Rochester) Strain of Virus.



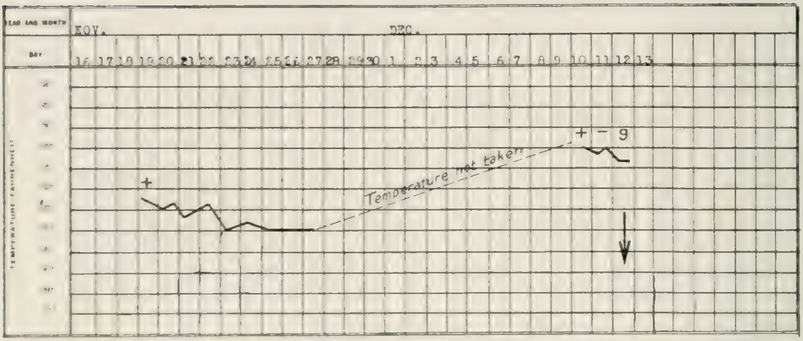
Remarks: November 16. Rochester pig. Not inoculated.
November 20. Symptoms of chronic type.
December 9. Animal found dead.
Lymphatic glands, lungs, spleen, heart muscle, and kidneys show typical lesions.

California (Hall) strain of virus.—Dr. Ivan C. Hall of California supplied this virus and the following history:

"We have just bled a hog originally injected with a virus obtained from one of our California towns. This virus was injected after being filtered and proven bacteria free, as it originally contained pure culture of *B. cholera suis*.

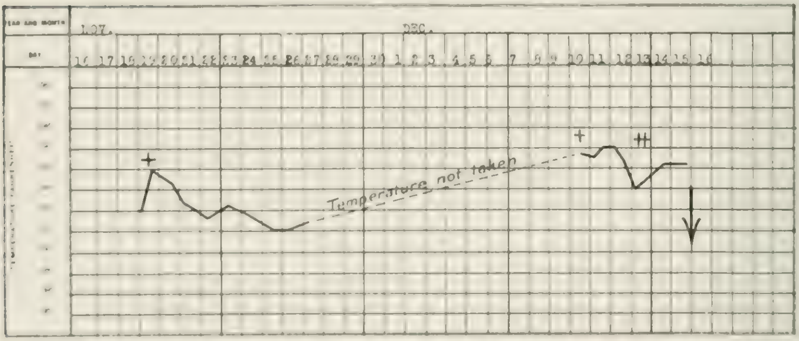
"The hog became very sick about 10 days after and, appearing moribund, we bled him to death. The virus which I am sending you is some of this, after it had been diluted to one part in four and filtered through a Berkefeld filter."

Hog No. 510. Michigan (Rochester) Strain of Virus.



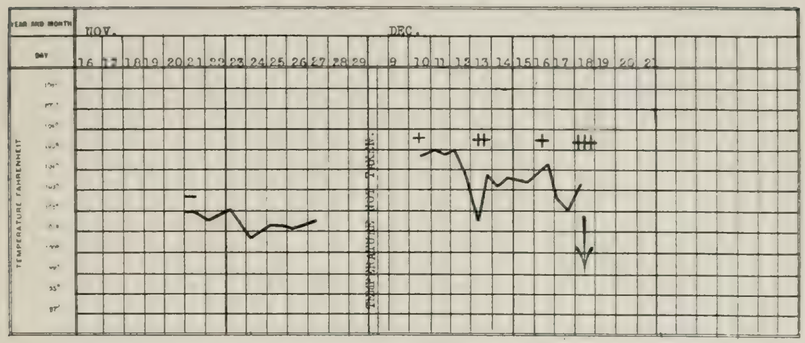
Remarks: November 16. Rochester pig. Not inoculated.
November 20. Symptoms of subacute type.
December 10. Animal very weak and emaciated.
December 12. Animal moribund, bled and examined.
Spleen, lymphatic glands, and cecum show typical lesions of cholera.

Hog No. 511. Michigan (Rochester) Strain of Virus.



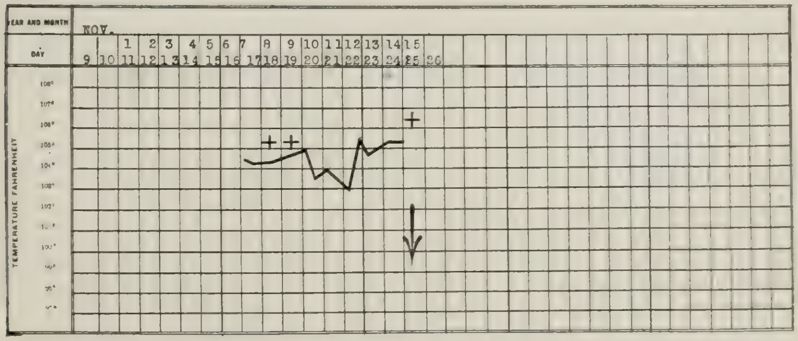
Remarks: November 16. Rochester pig. Not inoculated.
November 20. No marked symptoms, looks fairly good.
December 1. Symptoms of subacute type.
December 10. Animal very weak and emaciated.
December 16. Found dead.
Typical lesions, lymphatic glands, kidneys, lungs, and cecum.

Hog No. 513. Michigan (Rochester) Strain of Virus.



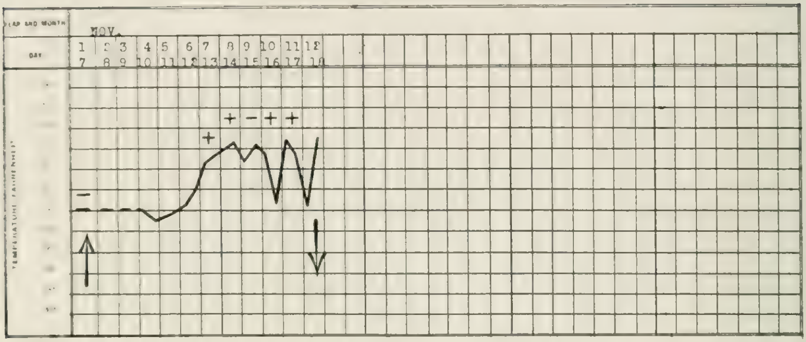
Remarks: Rochester pig, not inoculated.
November 20. No marked symptoms, but not doing well.
December 5. Subacute or chronic case.
December 10. Animal weak and emaciated.
December 18. Animal moribund, bled and examined.
Lungs, heart muscle, lymphatic glands, kidneys, and cecum show typical lesions.

Hog No. 514. Michigan (Rochester) Strain of Virus.



Remarks: November 9-11. Rochester pigs were placed with this hog, which was normal.
November 25. Animal moribund, bled and examined.
Typical lesions, lymphatic glands, kidneys, lungs, and spleen.

Hog No. 501. INDIANA I STRAIN OF VIRUS.

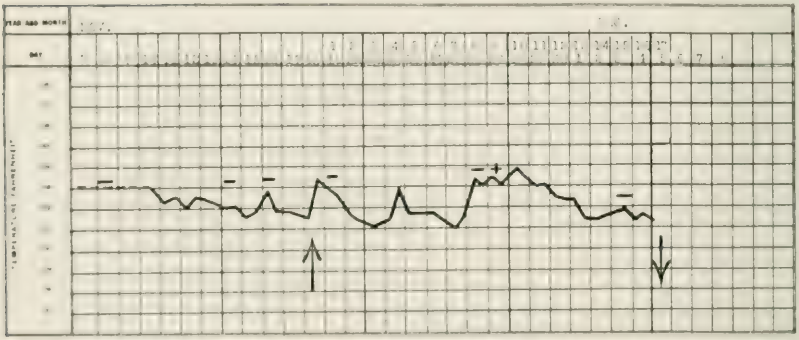


REMARKS: November 7. Inoculated with 5 c.c. of Indiana I Virus.

November 12. 4 P.M. Animal showed symptoms.

November 18. Animal moribund, bled and examined. Purple areas of discoloration on abdomen, nose, and ears. Subcutaneous blood extravasations. Lymphatic glands enlarged and hemorrhagic. Both kidneys petechiated and contain retention cysts. Hemorrhagic areas in heart muscle, lungs partially consolidated in both upper and lower lobes. Ulcers in cecum.

Hog No. 502. CALIFORNIA (HALL) STRAIN OF VIRUS.



REMARKS: November 18. Inoculated with 15 c.c. California (Hall) virus (diluted 1:4) and filtered through Berkefeld.

November 26-30. Slight symptoms.

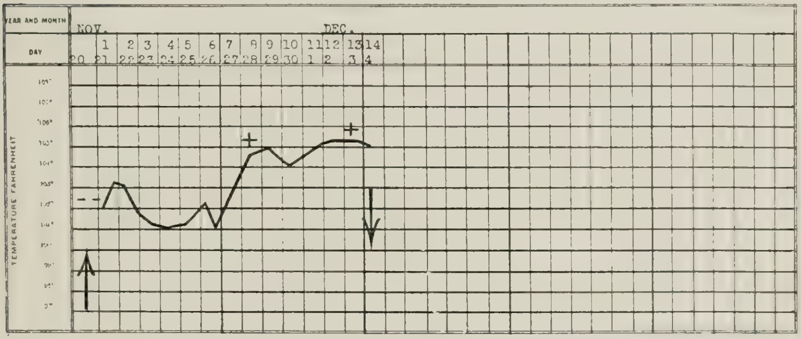
December 3. Animal apparently normal.

December 5. Animal killed by large male Hog No. 400. No lesions.

Hog 502, which received the above strain of virus, had been kept for several days as a control on the isolation of the experiments and on the dark field examinations of the blood of normal and diseased hogs. Nine days after inoculation this animal exhibited mild symptoms of hog cholera, with temperature of 103.5° F. The spirochete was found in its blood. Fifteen days after inoculation its blood was free from spirochetes and characteristic granules. On the 17th day it was killed by large male Hog 400, and at autopsy no pathological lesions could be found.

Ohio (Pettigrew, Hazen, and Heinz) strains of virus.—The viruses, obtained through the kindness of Dr. Paul Fischer and Dr. A. D. Fitzgerald, were taken from three different outbreaks and represented mixtures of blood samples from the number of pigs indicated on the labels. Ohio (Pettigrew), Ohio (Hazen), and Ohio (Heinz) represented mixtures or virulent blood from three, five, and six pigs respectively.

Hog No. 515. Ohio (Pettigrew) Strain of Virus.

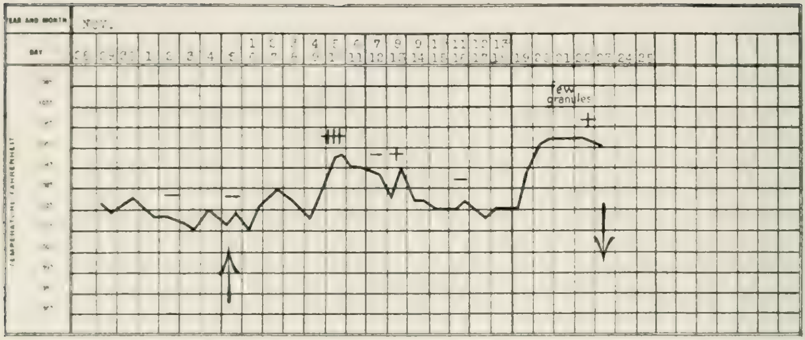


REMARKS: November 20. Inoculated with 15 c.c. filtered (Pettigrew) Ohio virus, diluted with normal saline 1-4.
November 29. Animal quite sick.
December 4. Animal moribund, bled and examined.
Spleen, kidneys, lymphatic glands, liver, lungs, and cecum show typical lesions.

A dark field examination was made of the undiluted Ohio (Pettigrew) virus, as found in the original container, and one spirochete was observed.

The spirochetes were found in the blood of hogs inoculated with all three strains of Ohio virus, as shown on the following charts. The blood of Hog 521 (Ohio, Heinz strain) on the fifth day after inoculation contained relatively numerous spirochetes. On the seventh day none could be found, while on the eighth day the organism was readily found. By the eleventh day the clinical condition was much improved and the findings were negative, but the animal suffered a relapse, and on the day of death the organisms were again present in the blood.

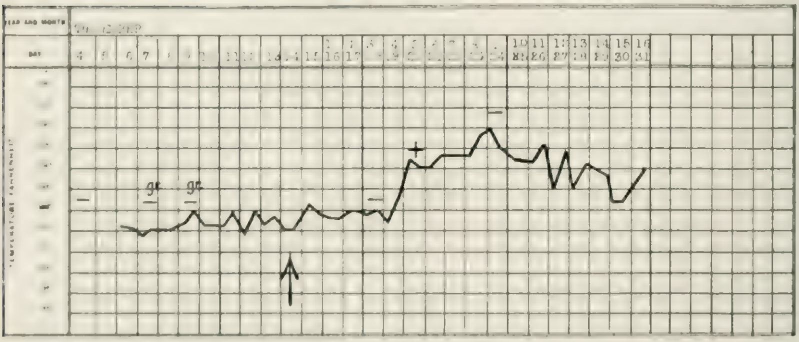
HOG NO. 521. OHIO (HEINZ) STRAIN OF VIRUS.



REMARKS: December 2. Hog in good condition.
December 5. Inoculated with 15 c.c. Ohio (Heinz) virus, Berkefeld filtered and diluted 1-4.
December 10. Symptoms appeared.
December 15. Condition of animal improving.
December 19. Animal suffered relapse.
December 23. Animal died.

Ventral surface of body deep purple color. Extravasations in subcutaneous tissue. Inguinal glands large and hemorrhagic. Lungs, both involved in all portions, highly congested, partially consolidated in small areas. Bronchial tubes filled with exudate. Heart, petechiae in auricles and the heart muscle. Both kidneys show small petechiae. Liver, passive congestion. Mesenteric and retroperitoneal and inguinal lymph glands enlarged and hemorrhagic. Cecum shows ulcers.

HOG NO. 525. OHIO (HAZEN) STRAIN OF VIRUS.



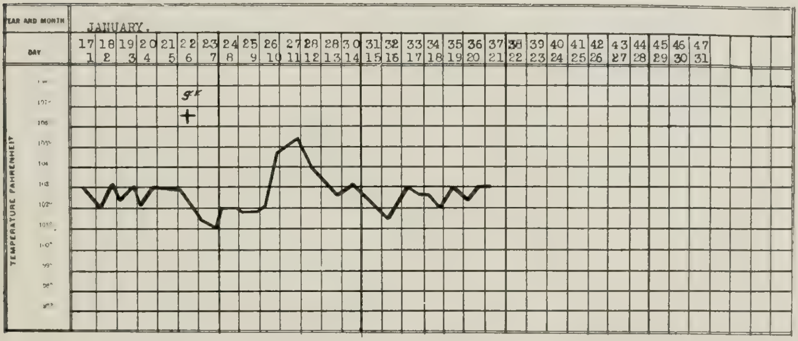
REMARKS: December 4. Susceptible pig; fine condition.
December 14. Inoculated 15 c.c. Ohio (Hazen) virus diluted 1-4 and Berkefeld filtered.
December 20. Anorexia, listless.

Hog 525, inoculated with the Ohio (Hazen) strain of virus, manifested symptoms on the fifth day after inoculation, and on that day the findings were positive. This animal is now fully recovered and no spirochetes are present in his blood.

Kansas I strain of virus.—Professor L. D. Bushnell supplied this virus and described it as follows: "I sent you a couple of ounces of hog cholera blood, which was collected from an outbreak about a mile east of Manhattan, Kan., by Doctor Gingery. This animal was killed and showed typical lesions. It had a temperature of 108°. Twelve animals had died when the herd was vaccinated."

This virus, when received, was examined on the dark field. It was found to contain numerous granules, some bacteria, but no spirochetes were observed. The blood of Hog 516 inoculated with this virus showed the presence of the spirochete.

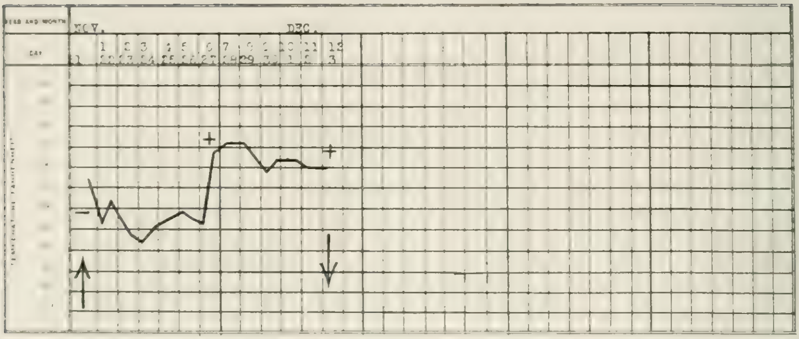
Hog No. 525—*Continued.*



REMARKS: January 21. Animal fully recovered, released.

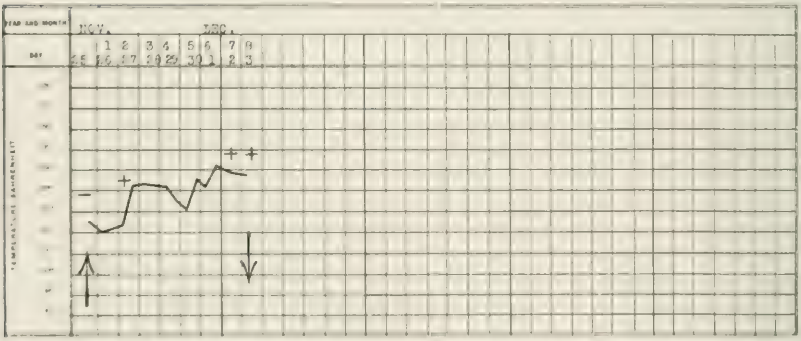
Unknown strain of virus.—On November 25 and 26, Hogs 517 and 518 were inoculated with California (Hall) and California (University) strains of virus respectively. Before inoculation, blood from these apparently normal animals were collected in sterile sodium citrate solution for control examination. In the case of Hog 518 the dark field examination of the assumed normal blood revealed the presence of the spirochete. Subsequent developments clearly indicated that these animals were in some way exposed to the disease before inoculation, as clinical evidences of the disease were clearly present in both cases within a day or two following inoculation, an interval of too short duration to correspond with the incubation period which should follow the injection of virus. Moreover Hogs 517 and 518 were both in a moribund condition, eight and seven days respectively, following the inoculation, periods relatively shorter than the average duration of the course of the disease of cases included in this work.

Hog No. 516. Kansas I Strain of Virus.



Remarks: November 21. Inoculated with 4 c.c. Berkefeld filtered Kansas I virus.
November 29. Animal very sick.
December 3. Animal moribund, bled and examined.
Typical lesions, marked in lymphatic glands, kidneys, cecum, spleen, and lungs.

Hog No. 517. Unknown Strain of Virus.

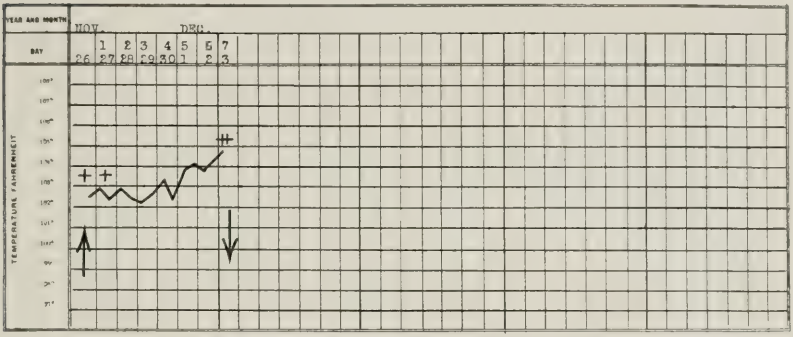


Remarks: November 25. Inoculated with 15 c.c. California (Hall) virus, Berkefeld filtered and diluted 1-4.
November 26. Animal shows symptoms, evidently due to previous natural exposure.
December 3. Animal moribund, bled and examined.
Cecum, lymphatic glands, spleen, kidneys, and lungs show typical lesions.

California (University) strain of virus. Dr C. M. Haring and Dr. F. J. Mitchell kindly furnished this specimen of virus. The history of the material follows: "This particular virus I am sending comes from an outbreak of a not severe type, but the virus has been killing pigs now in from seven to twelve days for the last four months. Pigs showed petechiae in the lungs and kidneys, very few on the intestines. Ulcers are sometimes present, sometimes not."

This strain of virus was injected into Hog 524 after having established three negative dark field examinations of his blood. As shown on the chart the spirochetes were observed at three different times during the course of the disease. Again, in this case, the spirochete was found about 24 hours before any clinical symptoms or rise of temperature occurred.

Hog No. 518. Unknown Strain of Virus.

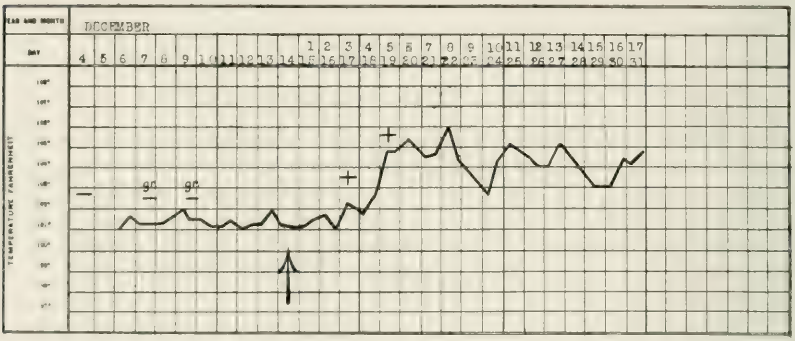


Remarks: Inoculated November 26, with 15 c.c. University of California virus, filtered and diluted 1-4.
November 28. Not eating well, red spots on ventral surface of body. Probably infected with natural exposure previous to inoculation.
December 3. Animal moribund, bled and examined.
Small blood extravasations in muscular and subcutaneous tissue. Spleen slightly enlarged, dark, gorged with blood. Capsules of both kidneys loosely adherent, and both kidneys filled with small urinary cysts. Both lungs show areas of consolidation. Inguinal and mesentery glands enlarged and hemorrhagic. Ulcers present in cecum.

Michigan (Rochester II) strain of virus.—On January 10, 1913, 15 apparently normal hogs were received from Rochester, Mich. These pigs, averaging about 40 pounds in weight, while not in thrifty condition, did not appear to be diseasesd.

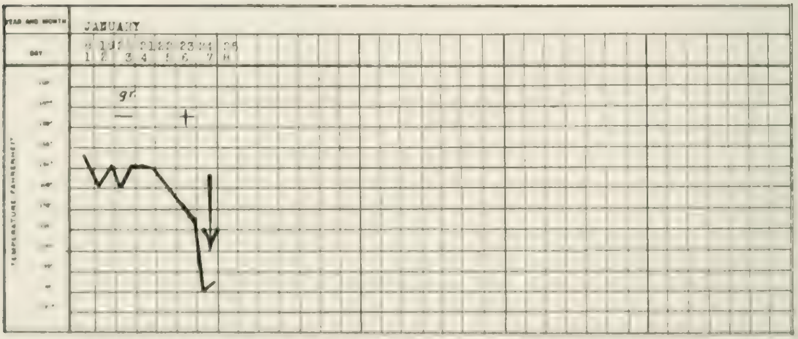
On January 11, dark field examinations of the blood of two of these animals revealed the presence of the spirochetes. The temperatures of these two animals were each 103.8° F. and a tentative diagnosis of the chronic type of cholera was made. On January 13, three days after the receipt at the laboratory, the temperatures of seven of the 15 animals ranged between 104° and 105.5° F. and, from other symptoms, there was no doubt but that the diagnosis of cholera was correct. Investigation showed that on January 1 these animals were placed under such conditions that exposure to hog cholera might result. As they were received at the laboratory on January 10 and symptoms definitely established by January 13, there existed a period of incubation corresponding to that usually present in the chronic or subacute types of cholera.

HOG NO. 524. CALIFORNIA (UNIVERSITY) STRAIN OF VIRUS.



REMARKS: December 4. Susceptible pig; fine condition.
December 14. Inoculated 15 c.c. California (University) virus, Berkefeld filtered and diluted 1-4.
December 18. "Off feed." Symptoms appeared.

HOG NO. 524—*Continued.*



REMARKS: January 8. Found dead.
Typical lesions, lungs, liver, kidneys, spleen, cecum, inguinal glands, large intestine, and subcutaneous tissue.

These animals are under observation at the present time. A summary of the dark field findings is presented in the following table:

TABLE 1.

MICHIGAN (ROCHESTER II) STRAIN OF VIRUS.

Hog.	Number Dark Field Examinations to Date.	Presence of Spirochete in Blood.	Remarks.
537.	3	+ + +	Temp range from 102 —104.8° F.
538.	3	+ + +	" " " 101.2—104.1 "
539.............	3	+ + —	" " " 102.2—105.5 "
540.............	1	—	" " " 101 6—103.5 "
541.............	1	+	" " " 101.6—103.2 "
542.............	3	+ + —	" " " 102. —104.6 "
543.............	1	+	" " " 102. —103.5 "
544.............	2	+ +	" " " 102.4—105.5 "
545.............	1	—	" " " 101.8—103. "
546.............	1	—	" " " 101. —104.5 "
547.............	2	— —	" " " 101.5—103. "
548.............	2	+ +	" " " 102. —105. "
550	1	—	" " " 101.5—103 "
551.............	1	—	" " " 102. —103.5 "
552.............	1	+	" " " 101.6—103 8 "

DARK FIELD EXAMINATIONS OF THE BLOOD OF NORMAL HOGS.

In addition to the control examinations which were made in practically all cases, as shown on the charts above, additional data relative to negative findings in normal blood have been secured.

TABLE 2.

DARK FIELD EXAMINATIONS OF THE BLOOD OF HOGS NOT SUFFERING FROM HOG CHOLERA.

Hog.	Number Dark Field Examinations.	Spirochete Present or Absent.	Condition of Animal.
522.............	2	— —	Normal
512.............	1	—	"
523.............	1	—	"
526.............	2	— —	"
519.............	2	— —	"
520.............	2	— —	"
527.............	3	— — —	"
528.............	1	—	Infested with Ascaroides
529.............	2	— —	" " "
530.............	1	—	" " "
531............	1	—	" " "
532.............	1	—	" " "
533.............	1	—	" " "
534.............	1	—	" " "
535.............	1	—	" " "
536.............	1	—	" " "
400.............	1	—	Natural immune
553.............	2	— —	Normal
554......	2	— —	"
555.............	2	— —	"
556.............	2	— —	"

A number of hogs, planned for material to be used in the work, became badly infested with intestinal parasites (chiefly *Ascaroides suella*) and were not utilized except for control blood examinations.

Some of these animals exhibited temperatures as high as 106° F. at the time of the dark field examinations. The negative findings in these cases, therefore, present valuable data in that they show that the presence of the spirochete, observed in the blood of animals suffering from cholera, does not depend merely upon a febrile condition of the blood.

Several immune hogs not included in the above data also served as donors of normal blood specimens. These additional negative findings in the blood of hogs not infected with hog cholera are shown in Table 2.

HORSE SERUM VIRUS PHENOMENON.

Horse serum virus may be produced by securing blood from a horse one to two hours after the animal has received an intravenous injection of approximately 150 c.c. of virulent hog cholera serum. The character of this material and the results of experiments, which demonstrate that it is not a mere dilution of hog cholera serum, have been reported in former publications.[1] It may be concisely stated that hog cholera virus undergoes some inexplainable process of activation after approximately one hour's residence in the circulatory system of the horse.

On October 25 the blood of Horse 1 was examined on the dark field. Nothing other than erythrocytes, leukocytes, and some blood dust was noted. At 1:45 P.M. on October 25, Horse 1 was given an intravenous injection (jugular vein) of 150 c.c. B.A.I. strain of virus. A specimen of this hog cholera serum was examined on the dark field and no spirochetes were found, although the material did contain many characteristic granules. The animal was led from the stocks and immediately became groggy and weak, the pulse was weak, respiration labored, eye dull and listless and peristalsis violently active. The animal became covered with a profuse cold perspiration and frequent watery evacuations followed. Three-fourths of an hour after the injection of the horse it was bled under aseptic conditions and the blood examined by dark field illumination. The spirochetes were found with ease, six organisms being found in a few minutes. Blood drawn from the horse one and three-fourths hours after injection also contained the spirochete when examined on the dark field. The one and three-fourths hours virus also contained a marked increase in the number of granules. A specimen of the blood of Horse 1 drawn two and three-fourths hours after injection upon careful dark field examination revealed only granules and refractive bodies—no spirochetes could be found.

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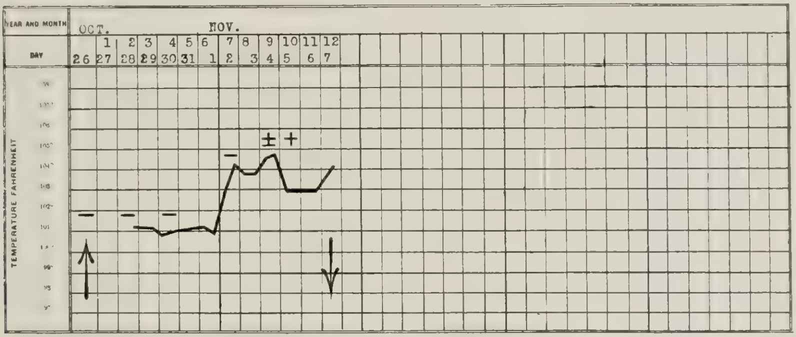
[1] King and Wilson, *Bull. Kansas Exp. Sta.*, 1910, No. 171. *Jour. Infect. Dis.*, 1912, 11, p. 441. *Ztschr. f. Immunitatsf. u. Exp. Ther.*, 1913, 16, p. 367.

The virulence of the three-fourths-hour horse serum virus which the above animal yielded was tested by the inoculation of Hog 4501. The result of this animal inoculation conformed with extensive data previously reported, in that the hog became the subject of a more acute type of cholera after a relatively short period of incubation, as compared with the average case following the injection of hog cholera serum itself.

HOG NO. 4501. B.A.I. STRAIN OF VIRUS. HORSE SERUM VIRUS.



REMARKS: October 26. Inoculated with 4 c.c. activated horse serum virus.
November 2. Symptoms appeared.
November 7. Animal found dead and examined.
Many subcutaneous blood extravasations. Muscle of right ventricle contains blood extravasations. Lower lobe left lung consolidated. Areas of red hepatization in upper lobes of both lungs. Spleen soft, dark, and gorged with blood. Kidneys show numerous petechiae. Large ulcer near ileocecal valve. Extensive areas of congestion in intestinal mucosa. Lymphatic glands enlarged and hemorrhagic.

On November 22, Horse 2 was utilized in duplicating the above experiment, after establishing a normal condition of the blood in so far as absence of spirochetes was concerned. This animal was injected intravenously with 180 c.c. B.A.I. and Indiana I strains of virus mixed. The symptoms were of the usual character, but were more retarded and less pronounced than those recorded in the first experiment with Horse 1. In our experience,[1] more pronounced reactions following injection and more uniform horse serum virus result, when the animals have been subjected to one or more repeated injections with the hog cholera virus, a condition in all probability not depending upon anaphylaxis as shown by previous results. Horse 1, above, had been repeatedly used on the work; Horse 2 had never been subjected to the treatment before.

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[1] Grateful acknowledgment is made to Dr. R. H. Wilson, who for several years has been associated with the senior author in this work, and who rendered valuable assistance in carrying out the horse serum virus experiments included in the present investigation.

The three-fourths-hour horse serum virus of Horse 2 showed the spirochetes on the dark field. In the two-hour virus only one spirochete could be found.

The above data must be verified by repeated experiments. However, assuming that the spirochete under observation, in different stages of its life cycle, might represent the etiological factor in hog cholera, it would not be impossible to explain the horse serum virus phenomenon by the results obtained above.

SUMMARY.

The results may be summarized as follows:

1. Dark field examinations of the blood of 48 hogs, all normal, excepting some which were infested with intestinal parasites, have revealed no spirochetes. All of these specimens have been relatively free from granules, with the exception of some instances, when it was usually possible to determine that the granules were liberated from crushed leukocytes.

2. The examination of the blood of all hogs—40 animals—which were suffering from hog cholera, revealed the presence of a spirochete. All specimens of blood from these animals, during the height of the disease, contained characteristic granules.

3. Negative dark field findings followed positive findings in the case of six hogs which recovered from the disease.

4. The blood of two naturally immune hogs was free from spirochetes and granules.

5. The spirochetes and granules have been observed in hogs infected with 12 different strains of virus, as follows: B.A.I., Michigan (Demerick), Michigan (Rochester I), Indiana I, California (Hall), Ohio (Pettigrew), Ohio (Hazen), Ohio (Heinz), Kansas I, Unknown, California (University), and Michigan (Rochester II).

6. Hog cholera was diagnosed in two small herds of hogs after receipt of the animals at the laboratory, by means of the dark field examination of the blood. In other instances, positive dark field findings were secured a few hours before any symptoms appeared.

7. The spirochetes were found with less difficulty in the blood of hogs suffering from the acute form of the disease.

8. In two experiments the spirochetes were demonstrated in horse serum virus.

9. Spirochetes are uniformly demonstrable in the intestinal ulcers of hogs dead from cholera.

CONCLUSIONS.

Without cultures with which to inoculate susceptible animals it is, of course, impossible at this time to definitely ascribe to the spirochete under observation any pathogenic properties or etiological significance. Nevertheless, careful consideration should be given to the results which are consistent and uniform throughout, and which, in some particulars, strongly suggest the relationship of this spirochete to hog cholera, as a causative factor. As this organism has not been previously reported and described the name *Spirochaeta suis* may properly be suggested.

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BACILLUS BRONCHISEPTICUS—ITS RELATION TO CANINE DISTEMPER.*

BY N. S. FERRY, M.D., DETROIT, MICH.

In January, 1911, Dr. J. P. M'Gowan, who was working on grants from the Carnegie Trust and Moray Research Fund in the laboratory of Dr. James Ritchie at the Royal College of Physicians, Edinburgh, published in the *Journal of Pathology and Bacteriology*, London (3), the results of some observations on a laboratory epidemic especially among dogs and cats, in which the animals presented the symptoms of the disease called "distemper." In the April number of the same Journal, as a continuation of his first article, he described the organism which was found constantly in all of the affected animals. Dr M'Gowan, however, evidently was not aware of the fact that several months previously in the *American Veterinary Review* (1), I had published my bacterial findings in canine distemper and had described in detail the same organism. It is also very interesting that Dr. John C. Torrey, Director of the Department of Experimental Pathology of the Cornell Medical School, New York City, who, in collaboration with Mr. Rahe, was working along practically the same lines at the same time, independently isolated the identical organism. In my second article, published in June, 1911 (2), in the *Journal of Infectious Diseases,* my work was described in full, and at the time the name *Bacillus bronchicanis* was given to the organism. M'Gowan isolated his organism from the dog, cat, rabbit, guinea-pig, ferret, monkey, goat and also man in one case. Torrey and I, however, limiting our work to the dog, went much more extensively into the subject than did M'Gowan. Torrey, although he carried on his work about the same time as M'Gowan, did not read his paper until April, 1912, at the American Association of Pathologists and Bacteriologists, owing to the fact that my first article was published before he had completed his experiments. He, therefore, continued his work, which, as yet, has not been published.

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*Presented to the Forty-ninth Annual Meeting of the American Veterinary Medical Association, at Indianapolis, August, 1912. From the Research Department of Parke, Davis & Co., Detroit, Mich.

During the first part of this year, while working on an epizoötic among guinea-pigs, rabbits and monkeys, I was able not only to isolate the same microörganism from the animals, but showed that the infection in guinea-pigs was probably general throughout the United States and was perhaps an important contributory cause of the scarcity of guinea-pigs in this country. In the last April number of the *American Veterinary Review* (6) this work was reported, and at that time the name of the organism was changed from *Bacillus bronchicanis* to *Bacillus bronchisepticus*, as it was, beyond a doubt, the cause of an infectious disease among many animals. The full report of this work, as well as other work of mine along this line, was published in the July issue of *The Veterinary Journal* of London (7) of this year.

M'Gowan, while he did some work on the dog, made his most valuable contribution to the subject with his work on other animals, especially cats (4). He was the first to show that the organism which I had previously proved to be the cause of distemper in dogs, gave rise also to pathological lesions in other animals.

Torrey's work was valuable from the fact that he went so exhaustively into his experimentation on dogs. As his work corroborated mine so fully, a review of his conclusions, which are also found in my article in the *Veterinary Journal* of London, will be given. He says: "The only microörganism regularly found was *Bacillus bronchicanis*, and its regular occurrence, its presence in overwhelming numbers in the respiratory tract in the great majority of cases, its appearance in the very earliest stages of the disease, and its progressive invasion and increase in numbers as the symptoms increased in severity leads one to conclude, from the bacteriological findings alone, that this bacillus is an important factor in the causation of the disease." "The strains of this organism, which were isolated from sixty-five cases, reveal an absolute uniformity in cultural reactions with the exception of one test. A few strains, 13 per cent, were encountered, which caused a marked reduction of nitrates to nitrites, but with 87 per cent there was no reducing action. All these strains are agglutinated to about the same degree by a serum immune to a single strain. The bacillus, accordingly, is not split up into sub-varieties or groups as is the *Bacillus dysentericus* or the *Bacillus coli;* but all strains comprised within a single species. This bacillus elaborates no exotoxin." He also says: "This bacillus finds its most favorable soil on the mucous membranes of the respiratory tract, for it invades, as a rule, the organs of the animal to only a slight extent. Of sixty-five positive cases it was present in the liver in 30 per cent, in the spleen in 20 per cent, in the kidney in 16 per cent, and in the blood in only 6 per cent." "By experimental inoculation with the *Bacillus bronchicanis* we have been able to induce typical attacks of distemper, with typical incubation period and symptoms complex, and from such experimental infections the *Bacillus bronchicanis* was recovered readily in pure cultures and in great numbers." Dr. Torrey produced the disease experimentally by blowing the organism or infected dust into the nasal passage with powder blowers. His conclusions were as follows:

"1. Dogs which are immune to natural distemper show no symptoms when exposed to experimental infection with *Bacillus bronchicanis.*

"2. Dogs actively immunized to *Bacillus bronchicanis* are immune to natural distemper.

"3. Dogs recovering from attacks of experimental distemper induced by this bacillus are protected on exposure to natural distemper.

"4. The *Bacillus bronchicanis* is the only cultivatable microorganism present with uniformity and in great numbers in the tissues and organs of distemper cases.

"5. Typical distemper may be induced in susceptible dogs by infection with pure cultures of the *Bacillus bronchicanis* under conditions simulating natural modes of transfer, and the bacillus may be recovered in pure culture from the organs of such experimental cases."

For the benefit of those who have not had access to all of these reports, and also those who still hold to the old belief of a filterable virus as the cause of distemper, the data of M'Gowan, Torrey and myself will be reviewed briefly showing the work upon which we based our conclusions, namely, that distemper was caused by a bacillus which could be grown on the ordinary culture media. The work of Carré, which was merely a short report of two or three pages, is extensively quoted by authors on veterinary subjects, and yet I have never been able to find in the literature where it has ever been corroborated under conditions which eliminated infection from outside sources. On the other hand, the bacillus in question has been isolated repeatedly by several investigators and the experimental work has been corroborated by all who have undertaken to repeat it.

In reviewing the work of M'Gowan, Torrey and myself the subject, for convenience, will be taken up under the following heads:

Primary Infection.
Agglutination Experiments.
Protective and Curative Inoculations.
Experimental Distemper.

Primary Infection.—From 29 laboratory dogs with symptoms of distemper M'Gowan found the organism in 27 cases. From 67 laboratory cats with symptoms he found the organism in 65 cases. From 6 rabbits he found it in 6 cases. From 3 guinea-pigs he found it in 3 cases.

M'Gowan says, speaking more especially with regard to dogs, "The disease I have been investigating, 'distemper,' is one limited primarily to the mucous membrane of the nose. From this situation it may spread secondarily to the frontal sinuses and the middle ear, from which it may go to the brain." "The disease may further spread to the conjunctiva, to the trachea, to the lungs, causing, however, in the last case, pneumonia and sometimes empyema." He also says, "Speaking for the disease I have been investigating, it is not of the nature of a septicemia." M'Gowan was never able to isolate the organism from the blood of any of his animals (5).

Torrey, on the other hand, working with dogs alone, found the organism in 65 cases out of 80. He says, "We have had about 80 cases in all, comprising all the stages of the disease from the incubation period to the postmortem, mild cases as well as severe." Torrey's cultural work was more thorough than M'Gowan's, for he made a routine examination of all the organs of every animal. In making the cultures, the animals were as a rule exsanguinated from the carotid, the blood and pericardial fluid cultured and small, but approximately equal portions of the apex and basal regions of the lung, the liver, the spleen and kidney and sometimes of the brain were emulsified and plated, the whole carried out according to Bail's method. Plate cultures were also made from the exudate of the eye, nose and trachea. Of 55 positive cases Torrey found the organism in the liver in 32 per cent, in the spleen in 20 per cent, in the kidney in 16 per cent, in the blood in only 6 per cent. Torrey observes that as a rule the bacillus finds a more favorable locality for its primary growth in the trachea than in the nose, which also corroborates my work. "From this point," he says, "the virus gradually extends into the larger trachea and finally into the bronchioles, when it generally incites a more or less extensive broncho-pneumonia." This also corroborates my work, for it was found that when the *Bacillus bronchisepticus* was isolated in pure culture from a diseased lung the condition was, as a rule, that of broncho-pneumonia. I have seen but a few cases of lobar or massive pneumonia from which the *Bacillus bronchisepticus* was isolated in pure culture.

Up to the time of the publication of my second article I had isolated the bacillus from 97 dogs at autopsy. In 68 cases it was isolated in pure culture, either from the respiratory tract or blood. From the first 68 cases of distemper (other than experimental cases) I found the organism in 12 out of 42 blood cultures (28.5 per cent). Torrey found it in 6 per cent and M'Gowan not at all. I attribute this apparent discrepancy possibly to variances in method. If the blood was at all fluid in the heart it was my custom to take from one to four cubic centimeters, or if not fluid, as much of the clot as possible, and plant in 50 Cc. of bouillon. It is a matter of experience that the greatest number of positive blood cultures are obtained when large quantities of blood are planted in correspondingly larger amounts of fluid culture media. In attempting to obtain cultures from the internal organs, Torrey macerated small portions of the tissues, from which he made plate cultures, while I planted a large piece of each organ in a tube of bouillon. While the former method has advantages which are obvious, yet I believe the latter method will give a higher percentage of positive findings. Experience with this organism has shown that plating directly from tissues of the animal does not always produce positive results.

My findings with other animals, as given in the *Veterinary Journal* of London, are as follows:

"Number of animals posted	93
"Of these there are:	
"Guinea pigs	75
"Rabbits	12
"Monkeys	6
"*Bacillus bronchisepticus* isolated from 86 animals	92.5%
"From trachea, 81 animals	94.2%
"From lung, 46 animals	53.5%
"From liver, 34 animals	39.5%
"From blood, 29 animals	33.7%
"Pure cultures from trachea, 74 animals	86%

"The points concerning the disease in these laboratory animals, which should be emphasized, are as follows:

"1. None of the guinea-pigs or monkeys showed any signs of an infection of the nasal cavity or the eyes.

"2. The infection was probably primarily in the larynx and trachea, as the organism was found in pure culture in those situations in nearly every case.

"3. The infection in these three species of animals was that of septicemia in the majority of cases, as the bacillus was isolated in pure culture in many of the cases from the heart's blood and abdominal organs.

"4. All the guinea-pigs and nearly all other animals suffered in the earliest stages of the disease with a profuse diarrhea.

"5. If the case was taken early enough, the *Bacillus bronchisepticus* could be isolated without much difficulty from the intestinal discharges, and often in nearly pure culture from the mucus of the small intestines, where congestion was most in evidence.

"6. Some of the rabbits showed an infection of the nasal cavity, and a few of the eyes, but many died with only an infection of the trachea and bronchi, and in nearly every rabbit the bacillus could be isolated from the blood.

"7. None of the monkeys showed an infection of the nasal cavity or eye, but did give evidence of an infection of the trachea and bronchi, and a septicemia, as the organism was found in pure culture in the trachea, bronchi and blood. One of these monkeys had a typical bronchial cough."

One of the most complete proofs of the relation of *Bacillus bronchisepticus* to canine distemper may be found in the accompanying chart. This chart represents an epizoötic which took place among a number of pups I was raising for experimental purposes. A dog infected with a typical case of distemper accidentally came in contact with the keeper of these pups, with the result that nearly all became infected. The pups were watched, and from the time the first sign of the disease made its appearance until death, every abnormal symptom was recorded. This chart was of value, for it threw more light on the bacteriology and symtomatology of the disease than I could have gathered from any other source of information. A glance at the chart will show the points which should be emphasized. From the dogs killed early in the disease the *Bacillus bronchisepticus* was isolated from the trachea in pure culture. From those killed later and others which were allowed to die natural deaths, secondary organisms were isolated, together with the primary organism, the *Bacillus bronchisepticus*. From those which recovered from the disease and were not vaccinated the bacillus was isolated in pure culture from the trachea, while those which were vaccinated, with one exception, did not show the organism.

The first symptom to appear was either a cough or convulsion, usually the convulsion. This was followed shortly by diarrhea. Then came the serous discharge from the nose followed by that from the eyes. Then appeared the purulent discharge from the nose, closely followed by the purulent discharge from the eyes. As soon as the purulent discharge from the nose and eyes was noticed the trachea was found to contain the secondary organisms as well as the primary. Dog 105 was the first to become infected, while 106 and 107 soon followed. The remainder of the dogs were probably infected from 105, as they were all running together in a large room. The period of incubation accordingly for the epizoötic was five days. I have found this to be about the average period of incubation, whether dogs were experimentally infected or had accidentally contracted the disease.

Agglutination Experiments.—The agglutination work was carried on extensively by all three of the experimenters, and all

105	106	107	108	109	110	111	116	117	118	119	120	114	112	113	115
1 Convul.															
2															
3 Cough	1 Convul. Cough	Cough													
4	2														
5	3				Convul.										
6 Very ill	4 Cough increased	Cough increased	1 Cough	Cough	Cough										
7	5	Diarrhea Skin erupt.	2 Convul.	Convul.		1 Cough	Cough	Cough		Cough	Cough	Cough	Cough	Cough	Cough
8 Killed	6		3	Diarrhea	Diarrhea	2 Diarrhea	Diarrhea		Cough						
	7 Killed	Killed	4 Killed	Nose (serous)		3		Diarrhea	Diarrhea	Diarrhea		Diarrhea	Diarrhea		
			5	Killed	Nose (serous)	4 Nose (serous)	Nose (serous)	Nose (serous)	Nose (serous)		Diarrhea	Nose (serous)		Diarrhea	
			6			5 Eyes (serous)		Eyes (serous)	Nose (purulent) Eyes (serous)	Nose (serous)	Nose (serous)		Nose (serous)		Nose (serous)
			7		Eyes (serous)	6	Eyes (serous)			Eyes (serous)	Nose (purulent)	Eyes (serous)	Nose (purulent)		Eyes (serous)
			8			7 Nose (purulent)	Nose & Eyes (purulent)			Nose & Eyes (purulent)	Eyes (serous)	Nose & Eyes (purulent)			
			9		Nose & Eyes (purulent)	8		Nose & Eyes (purulent)	Skin erupt.	Skin erupt.	Eyes (purulent)				
			10		Died	9 Eyes (purulent) Skin erupt.									
						21 Died	16 Died	17 Died	Recovered	Recovered	Recovered	Recovered	Recovered	Recovered	Recovered
B. bronchi	B. bronchi.	B. bronchi.	B. bronchi.	B. bronchi	B. bronchi Staph. and others	B. bronchi Staph.	B. bronchi Staph.	B. bronchi. Staph. and others	B. bronchi	B. bronchi	B. bronchi	B. bronchi.	Sterile	Sterile	Sterile
Cough, Convulsion, and Diarrhea				Nose	Eyes	Secondaries						Vaccinated			

came to practically the same conclusions, namely, that the reaction was specific for the bacillus, that serum from dogs spontaneously infected gave positive reactions, although not in as high dilutions as those experimentally infected, or those artificially immunized agains the bacillus, and that the serum from healthy dogs which had not previously suffered with distemper did not agglutinate the organism.

Torrey says, "Dogs which are sacrificed during the earlier stages of the disease and those which have succumbed or seem about to die from the infection have not, as a rule, developed any agglutinins for this bacillus. On the other hand, animals which are holding their own, are recovering or have recovered fully carry agglutinins in their blood which clump the *Bacillus bronchicanis* in dilution of from 1 in 25 to 1 in 500." "This tardy development of agglutinins," he thought, "may be due to the slight properties of the bacillus."

M'Gowan says: 1. "When an animal is immunized with a certain strain, a fairly high agglutinative power is developed in its serum against that strain. This agglutinative power never was higher in the cases examined than 1 in 120 and rarely above 1 in 60."

2. "In such an animal the serum generally has marked agglutinative properties for strains of the organism other than that with which the animal was immunized." "Here, although the organisms were of very different strains from that used for immunization, some five cats and some four dogs, the agglutination is almost as marked as with the organism used for immunization."

3. "Animals in which there is no evidence of distemper—*e. g.*, healthy animals brought into the laboratory and killed at once—show no agglutinating properties in their serum against the organism."

4. "Where an animal is suffering from distemper, and when the organism is found in it during life or postmortem, the serum of that animal may or may not agglutinate a strain of the organism."

In my agglutination experiments it was found that the serum from all dogs suffering with the disease gave an agglutination ranging from 1 in 40 to 1 in 800, while the serum from those artificially immunized against the organism gave an agglutination ranging from 1 in 800 to 1 in 4000. The test of one serum against several different strains ranged from 1 in 600 to 1 in 6000. Here again the difference in method may have had something to do with the great range between M'Gowan's results and mine. In all my experiments the following procedure, which was found after many trials to give the best results, was adhered to. The emulsion for the agglutination tests was made from 18-hour agar cultures, taken off in physiologic salt solution, to which was added enough formaldehyde to make a 1-per-cent solution. This emulsion was allowed to stand for five hours at room temperature, which time was sufficient to kill all organisms. The tubes were placed in the incubator and were examined at the end of 24 hours. Serum from dogs which had never suffered with the disease gave negative results.

Protective and Curative Inoculations.—As this paper was intended to show the relation of the *Bacillus bronchisepticus* to dog distemper, nothing will be said at this time concerning the vaccines made from the organism, except in so far as it aids in proving the specific nature of the bacillus. Relative to vaccines, M'Gowan carried on but few experiments, while Torrey makes no mention of his work along this line, except in his conclusion. He says, "Dogs actively immunized to *Bacillus bronchicanis* are immune to natural distemper." My work with vaccines made with this organism dates back to about June, 1909, when experiments were undertaken to immunize and treat dogs with killed and live cultures of the bacillus. A large number of experiments have been carried on from that time to the present with vaccines of different strengths, with one or more inoculations at varying intervals, and it is safe to say that the majority of pups can be protected, provided they are injected a certain time before exposure. It has been found that for practical purposes, three injections, with a few days' interval between injections, give the best protection. Lately, experiments have been carried on with pups only a few days old, while they are still nursing their mother, and so far no ill effects from the injections have been seen, while it would appear the large majority of the pups are protected. As these experiments are not finished it is impossible to draw any conclusions of value. If the results are favorable the benefits of this protection cannot be overestimated. Experiments conducted the past two or three years have shown that about 60 to 70 per cent of distemper dogs may be saved providing the vaccine is given early in the disease, and the cases are as carefully handled as they would be without the treatment. I believe also that better results are obtained with a vaccine made from several strains of the organism than from one. As I have never been able to produce the disease by subcutaneous inoculation, it has been a safe procedure in my hands, and with more lasting results, to immunize with suspensions of live organisms. For practical purposes, however, that method is out of the question.

Experimental Distemper.—It is an interesting, but an explainable coincidence, that all investigators who have championed various microörganisms as the cause of canine distemper have claimed to have produced typical distemper with their respective viruses. From the experience of M'Gowan, Torrey and myself it would seem that infections from outside sources play an important part in these results. Enough stress was not laid by others on protecting the experimental animals from outside contamination. This great source of error was recognized by the last-named investigators, who used the utmost precautions with all their experimental inoculations, and it was only after their work was thoroughly checked up that they were willing to concede the bacillus was the etiological factor in this disease. I will quote from both M'Gowan and Torrey on this subject.

Torrey says, "Most of the investigators who have isolated various microörganisms from distemper dogs have claimed to have produced the same disease with the bacillus which they have considered the etiological factor. It would seem, however, that with a few exceptions the importance of excluding contact infection, direct or indirect, in their experimental animals has not been given due consideration. In our experiments the dogs used for experimental infection have been kept in a different part of the building and far removed from all possible contact with cases of spontaneous distemper. They have been cared for by a special attendant who has kept away from all other dogs in the laboratory. These dogs, of which the history was known in most instances, were placed in sterilized cages and kept for at least 14 and generally over 20 days before any experimental infection was attempted, in order to eliminate any possible outside infection. After inoculation they were placed in a room never before used for dogs, in new cages and cared for by a special attendant. It has been claimed by Carré that this disease may be experimentally induced in dogs by a filterable virus. Under the conditions, however, just outlined, I obtained uniformly negative results with filtrates from scrapings of the nasal and bronchial passages of distemper dogs and filtered blood, although in earlier and less carefully controlled experiments some apparently positive infections occurred. In these cases, however, the possibility of accidental transmission of the virus from distemper cases could not be eliminated. Our experiments certainly indicate that canine distemper is not due to a filterable virus."

M'Gowan says, "Not one of those who have stated that they have found the organism of distemper seems to have appreciated the infectiousness of the disease, for there is no mention in their papers of particular or indeed any care having been exercised to insure complete isolation. Neglect to mention such precautions in such a case cannot be overlooked." "The lack of such precautions would explain also the regularity with which the various observers have produced the disease in animals by subcutaneous inoculation of their organism."

I will quote from my second paper showing also the precautions that we felt necessary to take in order to prove conclusively that we were dealing with the causative organism.

"In order to carry inoculation experiments to a successful termination and be sure that the dogs were not accidentally infected, three large rooms in buildings isolated from each other were used, each room being in charge of a different attendant. The pups used were taken directly from their mothers as soon as weaned. After making sure that the pups were in a healthy condition, they were at once given a bath, and then thoroughly soaked in a germicidal solution and placed in the incubation room. Here they were allowed to remain from three to six weeks under strict observation, in the charge of a man who kept away from other dogs. From the incubation room the pups were placed in another room, where they remained under observation at least three weeks longer before experiments were made. The floors and walls of these rooms were of cement, making it possible to wash and spray thoroughly with a germicidal solution as often as deemed necessary. The rooms were thoroughly disinfected and cleaned before the reception of each lot of pups and after each experiment. They were under lock and key and no one was allowed to enter except the attendant, laboratory assistants and myself. Following these precautions and with careful feeding and handling, one could be reasonably sure that all abnormal symptoms following inoculations were due directly and solely to the treatment. In a third room, in a building far removed from the first two, were kept all the convalescing as well as all sick dogs and others under observation and treatment. All autopsies were held in the laboratory, where cultures could be taken conveniently without fear of contamination."

In speaking of his experimental work Torrey says, "It has been our aim in these infection experiments to approximate as closely as was feasible natural modes of transfer of the disease. Without doubt the port of entry is the respiratory tract and the vehicle of transfer the discharge from the nose or throat either in a moist or dry state." "Scrapings from cultures of this bacillus were dried, partially pulverized and mixed with dry sterile dust. By means of insect powder blowers this infected dust was projected into the nasal passages and mouth of the experimental animal. After an incubation period of 3 to 13 days definite symptoms of the disease developed in 6 out of 7 of these animals, the attacks ranging as is the case in natural conditions from mild to very severe and fatal issues." "The symptoms developed included rhinitis and bronchitis with persistent cough, typical broncho-pneumonia, vomiting, bloody diarrhea, conjunctivitis, and in three instances the appearance of a typical pustular eruption of the skin."

In my second article was reported in detail the work carried on by me in order to attempt to produce the disease experimentally. Between 40 and 50 dogs were used and in 28 of these the disease was produced, ranging from the mild type to the very severe. In several instances, following typical symptoms of the disease, death was the ultimate outcome. Being led to believe from my previous autopsy findings that the primary seat of the disease was in the larynx or trachea, the organisms were introduced experimentally into the trachea by means of hypodermatic syringes, the medium of transfer being either bouillon or physiologic salt solution. In this way there was practically no chance of secondary or other pathogenic organisms accompanying the specific bacillus to its seat of possible infection. Any infection, accordingly, resulting in this region, from which this organism could later be isolated in pure culture, would be caused by the organism in question. Symptoms, therefore, accompanying and following this infection would, *a priori*, be the result of the infectious process set up by the organism. While this procedure has its advantages, the method used by Torrey, which simulates natural modes of introduction of the organism, was just as reliable and possibly of more value.

In recapitulating I will state that the *Bacillus bronchisepticus* was found by three investigators independently in different parts of the world to be the cause of canine distemper. The organism was isolated from the large majority of cases in pure culture, and was found to agglutinate serum from dogs suffering with distemper as well as those immunized and experimentally infected with the organism, while all normal controls were negative. Suspensions of both live and killed organisms were found to protect dogs from natural distemper, and dogs experimentally infected were found also to be immune to natural distemper. Typical distemper was induced, and the organism again isolated and grown in pure culture under conditions excluding outside source of infection, thus fulfilling Koch's dictums to the letter, and disproving thereby any theory which may previously have been held that the disease is due to a filterable virus, or a virus which is not able to be grown on the ordinary culture media.

LITERATURE.

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DRUG INFLUENCE ON EXTRASYSTOLES OF THE MAMMALIAN HEART.[*]

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INTRODUCTION.

Not infrequently disorders of the heart are but indications of a general disturbance having a common origin in some other part of the body. The term "functional diseases" is applied to these, whether they arise directly from the automatic mechanism of the heart or from some more peripheral cause. Interesting chapters on "functional diseases" of the heart appear in all the standard systems of medicine and in many monographs on cardiac pathology and treatment.[**] It is to such works that the reader is referred for the literature on the subject and for a general study of functional diseases of the heart, including extrasystoles—the immediate subject-matter of the present paper. Visceroptosis and pelvic pathology are believed to be capable of producing extrasystoles of the heart. Gastritis, constipation or distention of the stomach may underlie an obstinate cardiac condition. High blood-pressure, dilatation and inflammation of the heart are recognized as sources of stimuli for extrasystoles. The manner in which the heart is stimulated in these cases is not without question. The extrasystoles present in high blood-pressure probably arise from the mechanical stimulation. In extrasystoles following chronic constipation, evidence would indicate that the absorbed toxic material serves as the stimulus. However, extrasystoles have recently been produced experimentally by the stimulation of the nerve-supply to the heart,[†] so that the stimuli for extrasystoles, arising from pathological conditions foreign to the heart, may reach the heart reflexly through the nervous system. In all these conditions the heart itself may be excessively irritable.

When the exciting cause for extrasystoles is recognized and is amenable to treatment, the cardiac condition becomes insignificant. In many cases, however, the primary cause cannot be located and remains enigmatic. In others the cause is known, but still is not amenable to treatment. *In just such cases, a decrease in the excitability of the heart through the administration of therapeutic agents would tend to render the heart irresponsive to these stimuli.* But no direct experimental evidence has so far been presented, that we possess any drugs which, when administered, are capable of depressing the irritability of the heart and thus of any benefit to extrasystoles. For this reason a series of experiments upon this subject was instituted, the results of which are embodied in the following report.

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[*] The experimental part of this paper was carried out in the Research Laboratories of Parke, Davis & Company, Detroit.

[**] Hirschfelder: Diseases of the Heart and Aorta, p. 892. Kelly: Practice of Medicine, p. 419. Osler: Modern Medicine, IV, p. 270. Allbutt: System, VI.

[†] Rothberger and Winterberg (*Zentralbl. fuer Physiol.*, Vol. XXV, p. 189, 1911).

DESCRIPTION OF METHODS.

The apparatus employed was simple in conception and construction, but thoroughly dependable for the problem at hand,

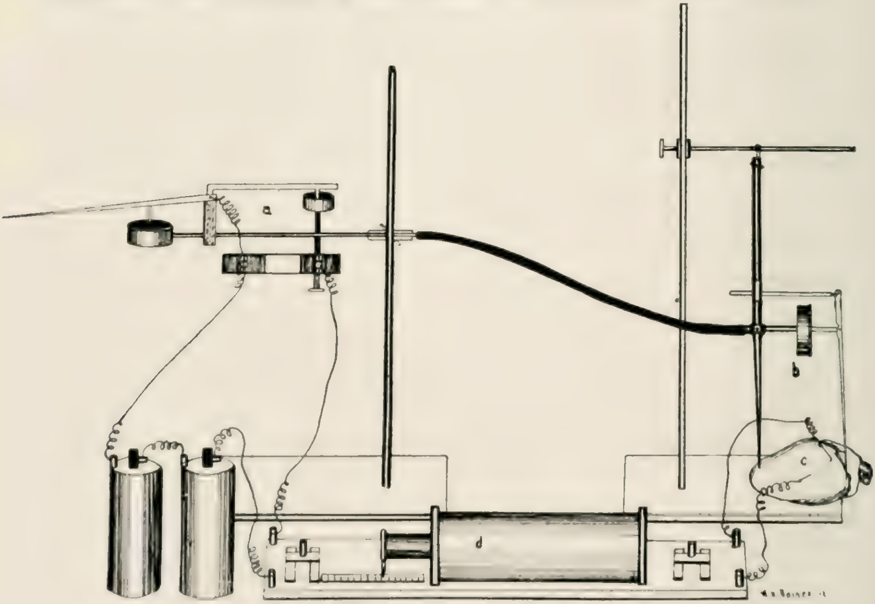

Fig. 1. Diagram of Apparatus.

(a) Extension lever and mercury cup.
(b) Cardiograph.
(c) Exposed heart with electrodes and cardiograph arms attached. Heart shown in diagram as though removed from animal.
(d) Coils and cells.

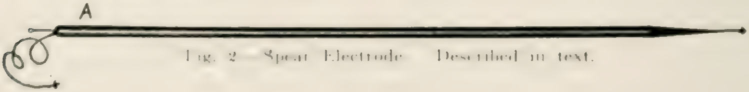

Fig. 2. Spear Electrode. Described in text.

as the control experiments to be described later will indicate. The general plan for the work was to have the heart automatically stimulate itself with single break shocks from an induction coil, thereby producing extrasystoles. The automatic cardiac stimulator used in this work was essentially that of Hirschfelder and Eyster.* Since the end to be attained was different in the two pieces of work, some modifications and adaptations were necessary. The writing lever of a recording tambour was extended backward 5 cm. This extension was of light metal, so as to permit the conduction of the current. To this extension (Fig. 1) was soldered a right angle arm of platinum, which dipped into a mercury chamber below. The mercury cup working on a screw could be raised or lowered, depending on the amplitude of the heart, and on the phase of diastole at which the stimulation was to occur. Connections from primary circuit were made with mercury cup and with extension lever. The connection with the extension arm was made at the pivot and was of tinsel-thread, to prevent spring action. From the secondary coil extended fish-hook electrodes which in open-chest experiments were applied directly to the ventricular muscle.

In closed chest experiments, small, straight, barbed spears were substituted for fish-hooks. These were insulated and strengthened by being run through the bore of small wooden tubes forming "spear electrodes" (Fig. 2). These were pushed directly through the chest-wall and into the muscle of the left ventricle, and served both for the conveyance of the stimulus and for registration, as will be indicated later. The fall in carotid pressure during the compensatory pause was so great and prompt that it could be taken as the indication of an extrasystole following stimulation. However, direct records of the heart were in all instances also taken. The amplitude record of the heart was traced by the writing lever of the stimulating tambour. This tambour was activated by the heart's contractions recorded through the cardiograph, a modified Marey tambour, the drawing of which makes its mechanism clear without further explanation. In open-chest experiments the two arms of the cardiograph were stitched to the ventricles and recorded the transverse shortening of the heart in systole. The writing lever of the automatic stimulator rose in systole and the extension dipped into the mercury chamber. In diastole the extension lever rose and broke the contact stimulating the heart in any desired portion of diastole depending on the height of the adjustable thumbscrew of the mercury chamber.

**Amer. Journ. Phys.*, Vol. XVIII, p. 222.

Two kinds of recording methods were used in closed chest experiments in which the respiration was natural. In the first, a long sound was introduced through the right jugular vein into the right heart. When properly placed the heart-end of the sound lay against the interventricular septum and against the anterior ventricular wall. The contractions of the heart made a working lever of the sound, the fulcrum being the muscles in the supraclavicular region. The free end was attached to the movable arm of the cardiograph, which activated the stimulating apparatus as before. In these experiments it was necessary to make a small incision on the left side for the application of fish-hook electrodes on the heart. In some cases artificial respiration was temporarily given, in others one lung with its pleura intact was ample for the short time (three minutes) that the chest was open. After application of electrodes the incision was rendered air-tight, and natural respiration was resumed. In this jugular sound-method of oscillations were fairly constant, but variations in the strength of contraction were not accurately recorded. This fact at first appeared as a disadvantage, but in some respects was an advantage, in that despite changes in amplitude, the heart was stimulated in the same phase of diastole.

Another more accurate method for recording cardiac contractions in closed chest experiments was found in applying the principle of Haycraft.* The spear ends of two spear electrodes, described before, were pushed through the left intercostal spaces and pericardium into the apex and base of the left ventricle. A trustworthy record of the heart's descent in systole was then obtainable by attaching the free end of the base spear electrode to the movable arm of the cardiograph. Tracings of amplitude changes, after administration of such familiar drugs as adrenalin, pituitary extract, barium chloride, were made as a test of the method, and compared exactly with tracings of the same drugs obtained in the more orthodox ways. In these closed chest methods shock was minimized, the vagus tonus was better, hemorrhage was avoided, and air irritation and drying of the heart were prevented.

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* [illegible] Vol. XIX, [illegible]

RELIABILITY AND LIMITATIONS OF METHODS.

Control Experiments.—If the heart be thus stimulated each time during the same portion of diastole, allowing thereby for the normal variations in irritability during different phases of diastole, then the strength of the secondary shock, just sufficient to induce an extrasystole, would be a criterion of the irritability of the heart at that time. Thus, increased and decreased irritability, as induced during an experiment, may be expressed in terms of the scale on an induction apparatus. The lowest portion of the secondary, just sufficient to produce an extrasystole, will be termed the "minimum stimulus" in this paper, and it should be constantly borne in mind that the irritability and the minimum stimulus bear an inverse relation to each other, *i. e.*, as the minimum stimulus increases, the irritability has decreased. Such an estimate of irritability may only be used with accuracy when we recognize the possibilities of irregularities in the strength of break induction shocks due to the apparatus itself, but fortunately the studies of Martin** allow one so to arrange the apparatus that this source of error is minimized. Accordingly, it may at once be stated that in all work attention was given to the following points. All throw of lever calculating for double sparking was as far as possible eliminated. The mercury surface was kept as clean as possible. The amount of sparking was lowered to a minimum. Break contact stimulations only were used. In experiments involving exposure of the animal's heart, a uniform moisture was maintained avoiding thereby resistance variations. Despite the fact that dry cells vary appreciably, especially when used over long periods, it was found that the primary current was constant enough for this work, provided the dry cells were changed frequently and disconnected, except for the time of actual tests.

In experimental work of this nature some normal variations must be recognized and proper allowance made in estimating

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[**] *Amer. Journ. Phys.*, Vol. XXII, p. 61, et seq.

results. It was noticed in some experiments that the heart gradually grew more irritable so that in normal animals the minimum stimulus gradually decreased. On account of this variation, experiments indicated that no test could be recognized unless the increase or decrease was at least 1.5 cm. difference in position of secondary coil above or below the minimum stimulus. In all tests sufficient time-interval was allowed between stimulations to prevent any change in irritability due to previous stimulation. The most critical test for the apparatus was that it was sensitive to the irritability charges during the different portions of diastole.

TABLE I.

Experiment B 12. July 1, 1911. Male Dog. Weight, 14 Kilo. Open Chest.

Time.	Minimum stimulus causing extrasystole.			Remarks.
3:37	6 —;	6.5+;	6.5+	First + indicates lowest position of secondary coil causing extrasystoles.
3:45	5.5—;	6—;	6.5+	
3:47	5.5—;	6—;	6.5—	
3:49	6—;	6.5+;		
3:55	6—;	6.5—;	7+	Heart moistened with salt solution before each test.
4:05	6—;	6.5+;	7+	

Experiment B 77. Sept. 5, 1911. Male Dog. Weight, 11 Kilo.

Time.	Minimum stimulus causing extrasystoles.			Remarks.
3:15	9—;	9.5+		
3:17	9—;	9.5+		
3:24	9—;	9.5+		
3:29	9.5—;	10+		
3:33	9.5—;	10+		
3:38	9.5—;	10+		
3:44	9.5—;	10+		New battery.
3:55	6.5—;	7+		
4:05	6.5—;	7—;	7.5+	
4:08	6.5—;	7+		
4:13	6.5—;	7+		
4:18	7—:	7.5+		
4:21	7—;	7.5+		

Changes of Irritability During an Experiment.—The two experiments tabulated below, carried out on normal animals, indicate the slight variations in the strength of secondary current sufficient to induce an extrasystole. In each test the heart was first tested with subminimal stimuli. Gradually the coil was moved toward the primary, until the "minimal stimulus" causing an extrasystole was found. After this the coil was moved back and the test repeated to make sure that the true "minimum stimulus" had been found. In all tabulations the first plus sign in each set of figures indicates the lowest position of the secondary coil sufficient to produce an extrasystole. The minus signs indicate at what positions of the secondary no extracontractions were elicited. In the second experiment the marked difference in the upper and lower portions were occasioned by a change of batteries as indicated in Table I.

Influence of Blood-Pressure on Irritability.—High blood-pressure has long been looked upon as a prolific cause of extrasystoles. As the blood-pressure varied during an experiment, it was determined to carry out a short series of experiments in which the aorta was clamped in its descending thoracic portion. This occasioned a very high pressure and spontaneous extrasystoles. The irritability was measured (1) before the compression of the aorta, (2) during the period of high pressure, and (3) after releasing the aorta. In every case the irritability was higher at the time of high pressure. The extrasystoles present at the time of high pressure disappeared on the releasing of the aorta. Any pressure-lowering drug also caused the disappearance of extrasystoles, only to reappear as the pressure resumed its former height.

Influence of Miscellaneous Agents.—No commensurable difference in the heart's irritability was noted in the change from natural to artificial respiration or vice versa. Hemorrhage, except in the extreme last stage just before death, caused no variations in irritability. Just before death from hemorrhage, the irritability became increased. Removal of the vagus influence, through section of both nerves, in all cases increased the irritability, but never beyond the limit of normal variation. Atropine in 1/60 gr. doses caused the same increase in irritability through paralysis of the vagus endings. This increase was constant but never more than 1 cm. difference in position of the secondary coil.

With these limitations in mind and guarded against, a study of drug influence on irritability was carried out, and the results are recorded in the next division of this paper.

TABLE II.

Position of Secondary Coil as a Measure of

	Irritability before compression. I.	Irritability during compression. II.	Irritability after releasing aorta. III.	Difference in irritability between I and II.	Remarks.
Experiment Aug. 12.	B. 58. Dog, 12 Kilo.	Closed Chest.			
Aorta clamped	16—; 16.5—; 17.5+	12—; 13+		4.5	
Aorta occluded	12.5—; 13+	11.5—; 12+; 13+		1	
Aorta released		12.5—; 13+	13—; 15—; 16+	3	New battery.
Aorta clamped and released	9—; 9.5—; 10+	6—; 7; 9+	7.5—; 8+	3	
Aorta clamped		7.5—; 8+	11.5—, 12+	4	
1/50 gr. Nitroglycerin					
Experiment Aug. 14.	B. 59. Male Dog.	15 Kilo.			
Aorta clamped and released		9.5—; 10+	10—; 10.5+	.5	
Aorta clamped and released	10—; 10.5+	8—; 9+	10—; 11—; 12+	1.5	
Experiment Aug. 15.	B. 60. Male Dog.	15 Kilo.			
Aorta clamped	10—; 11—; 12+	6—; 7+; 10+		5	
Aorta released		11—; 12+	12—; 12.5—; 13+	1	
Experiment Aug. 17.	B. 61.				
Aorta clamped and released	10—; 11—; 11.5+	9.5—; 10+	10—; 11—; 11.5	1.5	
Experiment Aug. 19.	Male Dog. 12 Kilo.				
Aorta clamped and released	9.5—; 10+	6—; 7+	9—; 10+	3	
Aorta clamped	9.5—; 10+	6—; 7+		3	

ACTION OF DRUGS.

Although the general trend of treatment for functional heart diseases is more toward hygienics than drug therapy, still the total number of drugs that find favor with reputable therapeutists in their treatment of this condition constitutes a large list. The action of none of these has been worked out with precision, but are prescribed purely empirically. It is from this class that drugs were selected for study, hoping to put their somewhat

TABLE III.
Potassium Iodide.

No. and Date.	Amount.	Administered.	Minimum Stimulus before drug.	Minimum Stimulus during drug action.	Remarks
B50 8/3	1 c.cm. 20%	Intravenous	7—; 7.5—; 8+	8—; 10—; 12—; 13+	First + indicates lowest position of secondary coil causing extrasystole.
B52 8/5	1 c.cm. 20%	Intravenous	8.5—; 9+	19—; 20—	
B53 8/7	1 c.cm. 20%	Intravenous	9 —; 9.5—; 10+	10—; 10.5—; 11+	
B53 8/7	1 c.cm. 20%	Intravenous	8—; 8.5+	8.5—; 9+	
B53 8/7	½ c.cm. 20%	Intravenous	8—; 9+	12.5—; 13+	
B55 8/9	3 c.cm. 20%	Deep Muscle	13—; 14+	15—; 17+	
B56 8/10	3 c.cm. 25%	Deep Muscle	11—; 11.5+	10—; 11.5+	
B56 8/10	3 c.cm. 20%	Stomach	11—; 11.5+	11—; 11.5+	
B57 8/11	¾ c.cm. 10%	Intravenous	5.5+; 6+	6—; 6.5—; 7.5+	
B60 8/15	¾ c.cm. 10%	Intravenous	6—; 7+; 8+	10—; 11—; 12+	
B68 8/15	1 c.cm. 10%	Intravenous	12.5—; 13+	12—; 15—; 17—; 17.5+	
B69 8/27	1 c.cm. 10%	Intravenous	9.5—; 10+	10—; 11—; 11.5+	
B70 8/27	1 c.cm. 10%	Intravenous	6—; 6.5—; 7+	8—; 8.5+	
B70 8/27	1 c.cm. 10%	Intravenous	8.5—; 9+	10—; 11—; 12+	
B71 8/29	½ c.cm. 1%	Intravenous	8—; 8.5+	7.5—; 8.5+	
B71 8/29	1 c.cm. 1%	Intravenous	6—; 7+	6—; 9+	
B72 8/30	½ c.cm. 4%	Intravenous	6.5—; 7+	7—; 8—; 8.5+	
B72 8/30	1 c.cm. 4%	Intravenous	7—; 7.5+	7—; 8—; 8.5+	
B73 8/31	1 c.cm. 4%	Intravenous	6—; 6.5+	6.5—; 7+	
B74 9/1	1 c.cm. 4%	Intravenous	10—; 10.5—; 11+	10—; 12—; 12.5+	

TABLE III—Continued.

Barium Chloride.

No. and Date.	Amount.	Adminis-tered.	Minimum Stimulus before drug.	Minimum Stimilus during drug action.	Remarks
B17. 7/12	2.5 c.cm. 1%	Intravenous.	7—; 7.5+	8.5—; 9+	Typical action of barium on heart and blood pressure.
B19. 7/15	2 c.cm. 1%		4—; 5+	7—; 8—; 8.5+	
B21. 7/16	2 c.cm. 1%		5.5—; 6+	6—; 6.5—; 7+	
B22. 7/17	1.5 c.cm. 1%		8.5—; 9+	9—; 9.5+	
B26. 7/20	1 c.cm. 1%		4.5—; 5+	8—; 12—; 14—; 20—	
B30. 7/22	1 c.cm. 1%		10.5—; 11+	12—; 15—; 17—; 18+	
B50. 8/3	1 c.cm. 1/10%		12—; 12.5+	13—; 15—; 16—; 17+	
B50. 8/3	1 c.cm. 1/100%		11.5—; 12+	11.5—; 12+	
B53. 8/7	3 c.cm. 1%		8.5—; 9+	9—; 10—; 11+	
B69. 8/25	1 c.cm. 1%		9—; 9.5—; 10+	10—; 12—; 13+	
B71. 8/29	1 c.cm. 1%		5.5—; 6+	6—; 8—; 9+	
B72. 8/30	1 c.cm. 4%		7—; 7.5+	7—; 8—; 9—; 10+	
B73. 8/30	2 c.cm. 4%		3—; 4+	4—; 4.5+	
B78. 9/6	2 c.cm. 4%		4.5—; 5+	5—; 6+	
B83. 9/11	2 c.cm.		6—; 7+	7—; 8—; 9—; 9.5+	
B83. 9/11	2 c.cm.		5—; 5.5+	6—; 8—; 10—; 12+	
B84. 9/12	2 c.cm.		9—; 10+	10—; 11+	
B85. 9/14	2 c.cm.		8—; 8.5—; 9+	10—; 11—; 11.5+	

Chloretone.

No. and Date.	Amount.	Adminis-tered.	Minimum Stimulus causing extra sys-tole before drug.	Minimum Stimulus causing extra sys-tole during drug action.	Remarks.
B94 9/22	2 gr.	Intravenous	13.5—; 14+	14—; 15—; 16—; 16.5+	Closed chest. Dissolved in de-fibrinated blood.
B95 9/22	2 gr.	Intravenous	13.5—; 14+	14—; 15—; 16+	"
B95 9/22	2 gr.	Intravenous	13—; 15.5+	13—; 14—; 15—; 16+	"
B96 9/23	2 gr.	Intravenous	12.5—; 13+	13—; 13.5—; 14+	"
B99 9/27	2 gr.	Intravenous	10.5—; 11+	11—; 12—; 13—; 14+	"
B101 9/29	2 gr.	Intravenous	12—; 12.5+	13—; 14+	"
B101 9/29	10 gr.	Stomach	11—; 11.5+	12.5—; 13+	Dissolved in alcohol.
B105 10/3	2 gr.	Intravenous	14—; 15+	15—; 17—; 18+	Dissolved in blood.
B105 10/3	2 gr.	Intravenous	13—; 13.5+	14—; 15—; 15.5+	"
B107 10/5	2 gr.	Intravenous	5.5—; 6+	8—; 8.5+	Dissolved in alcohol.
B108 10/6	2 gr.	Intravenous	8—; 8.5+	8.5—; 9+	Dissolved in blood.
B108 10/6	2 gr.	Intravenous	8—; 8.5+	8.5—; 9+	"

obscure beneficial action on a more rational basis. To this list there were added those drugs that have been found to affect the irritability of the heart of cold-blooded animals.*

In this series of experiments it was found that many animals were very much more susceptible than others. The irritability could not be influenced at all in some few cases. Consequently no drugs were absolutely constant. And further, it was noted that in the open-chest experiments, the exposure of the heart, with its concomitant deleterious influences, rendered the heart much less sensitive to drug influences, and would thereby vitiate results unless proper consideration were given this point. With this in mind the drugs investigated may be placed in three classes, according as their action is pronounced and constant (Class I.), slight and uncertain (Class II.), entirely absent (Class III.).

Class I.—Action pronounced and constant. The most nearly constant results were obtained from the salts of potassium and barium, and from chloretone. (See table III.).

Discussion of Results.—From these tables it appears that on the administration of potassium iodide, barium chloride, or chloretone, the heart is rendered much less susceptible to electrical stimuli, so that a decidedly stronger current is required to induce extrasystoles. That is, the heart's irritability is depressed. Of these drugs, barium chloride augments the heart, chloretone depresses, and potassium iodide has little or no action in the dosage employed, and yet, different as they act upon the amplitude of the heart, they all depress the heart's irritability. Therefore the term "depression of the heart" in itself means little unless qualified in some such way as to mean depression of irritability or depression of contractibility, etc. Further, as seen in the case of potassium iodide, it is quite possible to alter one of the fundamental functions of the heart without any disturbance of the others. And in the case of barium, an inotropic influence is exerted in a positive way, while a bathmotropic influence is exerted in a negative way.

Class II.—Action slight or uncertain. In a second group fall a number of drugs, whose excitability-lowering action on the heart was either so small or so inconstant as not to be trustworthy. It was found in some susceptible animals that strych-

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*Schultz (*Amer. Journ. Phys.*, Vol. XXII, p. 133).

nine sulphate had a pronounced action in lowering the irritability. In the majority of cases, however, it was without influence. (See table.) The calcium salts almost constantly lowered the excitability, but to a very slight degree. Chloral hydrate in favorable experiments decreased the excitability, but owing to the marked depression of the heart the automatic stimulation was interfered with, so that these results have been disregarded. As was stated in an earlier portion of this paper, nitroglycerin decreased the irritability, but this action was not through any action on the heart, but through the lowering of the blood-pressure.

TABLE IV.

Strychnine Sulphate.

No. and Date.	Amount.	Administered.	Position of coil for minimum stimulus before administration of drug.	Position of coil for minimum stimulus during drug action.	Remarks.
B30. 7/21	1/30 gr.	Intravenous.	11.5—; 12+	12—; 13+	The first + indicates position of secondary coil giving an extrasystole.
B50. 8/3	1/30 gr.		12—; 12.5+	13—; 13.5— 14+	
B50. 8/3	1/30 gr.		10.5—; 11+	10.5—; 11+	
B53. 8/7	1/30 gr.		8—; 9+	10.5—; 11+	
B53. 8/7	1/30 gr.		8.5—; 9+	11—; 11.5+	
B55. 8/9	1/30 gr.		4—; 4.5+ 5+	7—; 8—; 9+	
B56. 8/10	1/30 gr.		10.5—; 11+	10.5—; 11+	
B70. 8/27	1/30 gr.		8.5—; 9+	8—; 8.5+	
B71. 8/29	1/30 gr.		5.5—; 6+	6—; 5.5+	
B72. 8/30	1/30 gr.		7—; 7.5+	6.5—; 7+	
B79. 9/7	1/30 gr.		8.5—; 9+	11—; 12—	
B81. 9/9	1/30 gr.		6.5—; 7+	7—; 7.5—; 8+	
B83. 9/11	1/30 gr.		6—; 6.5+	6.5—; 7+	
B84. 9/12	1/30 gr.		7.5—; 8+	7.5—; 8+	
B92. 9/22	1/30 gr.		8—; 8.5+	8.5—; 9+	
B93. 9/23	1/15 gr.		12.5—; 13+	13—; 13.5+	

Class III.—Action entirely absent. A third group of drugs is made up of those which were uniformly without action in decreasing the irritability. Atropine was found to have a slight action in increasing the excitability. This is true in the same minor degree of preparations of digitalis. Apparently none of the therapeutic value of digitalis is derived from decreasing the heart's excitability. Adrenalin and pituitary extracts have no effect except when used in such large amounts as to cause a very high pressure. This is of interest as showing that a drug may increase (adrenalin) or decrease (pituitary extracts) contraction and yet leave the irritability entirely unmodified. Morphine, cocaine, copper salts, ergotoxine and strontium were all without influence upon the excitability of the heart, in as far as we were able to detect with our apparatus in a limited number of injections of each drug.

SUMMARY.

1. Results favor Engleman's theory that one function of the heart may be modified without a corresponding modification of the others. Thus (*a*) potassium iodide depresses irritability, no effect on the contraction;

(*b*) barium chloride depresses irritability, increases contraction;

(*c*) chloretone depresses irritability, decreases the contraction;

(*d*) adrenalin, no change in irritability, increases the contraction;

(*e*) pituitary, no change in irritability, decreases the contraction.

2. Potassium iodide, barium chloride and chloretone, all depress the excitability of the heart. It must be borne in mind, however, that in the present stage this is of pharmacological rather than of therapeutic interest, for barium salts are contraindicated for any practical use on account of their poisonous nature, and the action from potassium iodide is not observed on administration *per os*.

3. In high pressure the irritability of the heart is much increased. Therefore, pressure-lowering drugs are serviceable in abolishing extrasystoles in high blood-pressure cases.

THE EMPLOYMENT OF PROTECTIVE ENZYMES OF THE BLOOD AS A MEANS OF EXTRACORPOREAL DIAGNOSIS.

I.—SERO-DIAGNOSIS OF PREGNANCY.

By Carey Pratt McCord, M.D., Detroit.

On the parenteral introduction into the blood of substances in any wise different in structural form from such as normally occur, there arise enzymes capable of disintegrating these foreign materials and transforming them into forms not qualitatively different from normal blood constituents. In short, parenteral digestion takes place. The portal of entry for such materials is from overloading the intestinal tract so that some food passes through the enteric barrier in complex form, from intravenous and intra-abdominal injections, or from the organs of the body that, from their individual specific nature, when thrown in the circulation as unchanged albumins are no less foreign than parenteral injections from without the body.

This formation of protective enzymes then is sensitization, and all the phenomena of anaphylaxis and immunity are closely allied. In this phenomenon is the basis for all prophylactic vaccination and the foundation for the curative activity of such substances as tuberculin. Although physiological in its nature, this disintegrating of stable proteins is not without its deleterious action as evidenced in the autointoxications.

Such an enzyme cleavage of proteins underlies the various cutaneous reactions, such as lepro-diagnosis, tuberculin reaction and the cutaneous diagnosis of syphilis. These phenomena come as a result of introduction and cleavage of substances of a kind like unto that with which the organism is sensitized. Not only will this cleavage take place within the body, as in the instances cited, but the drawn blood contains the active enzymes, and when placed in contact with substances against which they were generated, structural disrupting occurs extracorporeally, with a breaking down into simple forms similar to that occurring intracorporeally.

This field opens up unlimited possibilities for usefulness in diagnosis. The excellent work of Abderhalden (1) has developed many interesting facts which are likely to prove useful to both the clinician and the technical worker. Abderhalden's personal attention has been given largely to the sero-diagnosis of pregnancy, which is based wholly on the principles appearing above. His methods are, however, applicable to many phases of the same field. These methods, with modifications of our own, have been successfully employed in the present experimental laboratory study of the sero-diagnosis of pregnancy and the sero-diagnosis of toxic goitre. The paper deals only with the sero-diagnosis of pregnancy, and will be followed by a second paper on the diagnosis of toxic goitre.

THE SERO-DIAGNOSIS OF PREGNANCY.

The utility of such a method of diagnosis of pregnancy, if found trustworthy, is too obvious to need comment. It has long been known that the placenta, in its developing stage and period of growth in particular, discharges into the maternal circulation microscopic portions of the chorionic villi. The nausea, vomiting and other signs of intoxication are probably concomitant to the entrance of these extraneous placental portions. On their appearance in the circulation, enzymes relatively specific against chorionic villi are mobilized by the body, to which the name "choriolysins" has been given. It is around this phenomenon that the work centers.

EXPERIMENTAL METHODS.

Preparation of placenta. The placentæ of humans, cows, dogs and guinea-pigs were used. The placentæ were obtained at earlier stages than complete gestation, save in the case of women. These were obtained from normal patients in normal labor. The placentæ were freed from blood completely. Flushing through the umbilical vessels removes much of the blood at once. The fœtal membranes and cord were removed and discarded. The removal of the remaining blood content was facilitated by cutting into small portions and placing in a sieve under a running tap. On complete freeing from blood, the placentæ were placed in boiling water in a porcelain dish and boiled. Contrary to the experience of Abderhalden, we found that boiling for two five-minute periods was rarely sufficient. After boiling for five minutes the water was drained away and the tissue rinsed thoroughly and reboiled for a period of ten minutes. A 5 cc. sample of the boiling fluid was tested, in the manner described by Abderhalden and later in this paper, for non-coagulated protein. If positive, the process of changing water and boiling was repeated until the test was negative. Twenty to thirty minutes of actual boiling was necessary on the average. The placenta and fluid were poured into a large-mouth bottle and covered with toluol and tightly covered. This preparation was kept in the cold room to prevent deterioration.

Desiccated placental preparation. We found a more permanent and more easily handled preparation of placenta was to be obtained in the following way: The placentæ were boiled (after being washed free of blood) in the manner described above until the fluid on boiling with the reagent was negative. Toluol was added. The placental pieces were then rapidly ground to a fine paste in a Latapie grinder, and repeatedly extracted with pure acetone until the acetone remained uncolored from the placenta. The acetone was separated from the placenta by filtration through a Buchner funnel with exhaust. The placenta was dried in vacuo in an atmosphere of toluol vapor at room temperature. On complete drying, the placenta (at all times protected from contamination) was ground to a fine powder. The finished product is a fine, nearly white powder. This was used in the same way as the coagulated placenta, and by comparison with it in the same cases was found equally trustworthy.

Obtaining of blood serum. The serum must be obtained from the patient within a few hours prior to making the test. At least 3 cc. of clear serum is necessary, so that from 6 to 10 cc. of blood must be drawn. The serum must be free from hæmoglobin. The hæmoglobin itself is probably non-consequential, but its presence indicates the disrupting of blood cells, with the liberation of enzymes capable of vitiating the true condition. To obtain perfectly clear serum offers considerable difficulty. In animal work it was comparatively easy to obtain such a serum by drawing blood directly into small centrifuge tubes and quickly throwing down the cell content before coagulation occurred. Of this the upper half is usually a clear, hæmoglobin-free coagulum. By placing a glass bead on this and recentrifugalizing, a clear serum is expressed. In actual clinical work, clear serum usually may be obtained by drawing the blood from a vein of the forearm of the patient with a syringe, as for a Wassermann test. The blood is transferred immediately to small 5 cc. tubes and centrifugalized as described above, if suitable centrifuge is available. If no centrifuge sufficiently powerful to throw down blood corpuscles is at hand, the serum may be obtained by transferring the blood from the syringe to 5 cc. paraffined tubes packed in snow or ice. The blood thus kept cold is delayed in its coagulation so that the corpuscles tend to settle to the bottom. When coagulation does occur, the upper half again is relatively free from hæmoglobin, so that on standing a serum is spontaneously expressed that is usually free from hæmoglobin.

Dialyzing apparatus. The most prolific source of error arises from the dialyzing membranes. Abderhalden employed fish bladder kondoms in his original work, and later used and recommended the parchment thimbles of Schleicher and Schull. In our work collodion thimbles of about 20 mm. diameter were employed in a satisfactory way, except that it is difficult to obtain these collodion sacs of equal thickness so as not to interfere with the rate of dialysis. The parchment tubes were open to the same objection. Each tube had to be tested for weak spots and pin holes. Some tubes were entirely too thick, while others were too thin. These parchment tubes, after elimination of such sources of error, were by far the most serviceable and dependable of the forms of dialyzing apparatus investigated. These, with proper cleaning, may be used repeatedly.

The triketohydrindenhydrat reaction. This reagent was the chemical test for the dialyzation products. The material used was in part the market substance of Meister Lucius and Brüning, and in part supplied by Dr. V. L. King of this laboratory, who in addition has supplied the data for the description of the chemical nature of the substance.

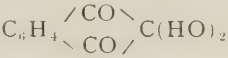
To detect the digestion products of proteins (placenta) in Abderhalden's biochemical test for pregnancy, a reaction was required which was extremely sensitive. In the digestive process albumin is degraded gradually, so that under the conditions of the test, only the dialyzable products being separated, peptones and amino-acids are the substances to be detected. The biuret reaction, which was discovered by Rose (2), is a method of detection of soluble substances possessing $NH(CONH_2)_2$, $(CONH_2)_2$, $CH_2(CONH_2)_2$, acid amide groups, when two $—CONH_2$ groups are connected by a N atom, a C atom, or directly to one another. The peptones contain two such groups and give the biuret reaction very strongly. As the digestion proceeds *in vitro*, however, the peptones are converted still further to the amino-acids and hence the solution to be tested will contain largely α-amino-acids rather than peptones. The ideal reagent, therefore, is one which is specific for α-amino-acids. From among the numerous substances available, Abderhalden found indantrione hydrat (triketohydrindenhydrat) to give the most reliable results. The amino-acids resulting from the digestion of albumin are always α-amino-acids. No other kinds have ever been found. Indantrione hydrat reacts only with the α-acids, although it also reacts with peptones and all substances containing acid amide groups to form soluble violet dyes. The solutions are dichroitic and appear red in transmitted light. The indantrione has become available through the researches of Ruhemann (3) and Perkin (4). It is prepared from cinnamic acid according to the following reactions:

$C_6H_5CH:CHCOOH$ ——— $C_6H_5CH_2CH_2COOH$
Cinnamic acid reduction Phenyl propionic acid

$C_6H_5CH_2CH_2COCL$
Phenyl propionic acid chloride

Friedel-Crafts synthesis $C_6HC_4H_2CH_2CO$
Indanone

Condensation with p-nitroso dimethyl anilin to a substituted anil and subsequent hydrolysis.



$$C_6H_4 \begin{array}{c} \diagup CO \diagdown \\ \diagdown CO \diagup \end{array} C(HO)_2$$

Indantrione hydrat

The test. One-half gram of the coagulated placenta or ¼ gram of desiccated placenta is placed in the parchment thimble. This thimble should be washed thoroughly just prior to use. Add to the placenta in the tube 1½ cc. of serum free from hæmoglobin. Avoid any contact of placenta and serum with outside of thimble. As a further precaution rinse thoroughly on the outside. Place in dialyzing jar containing 10 to 20 cc. distilled water. A large test tube makes a suitable dialyzing jar. Add a layer of toluol to the surface of the water and to the thimble contents to prevent deterioration. In like manner prepare a control test in all respects similar except no placenta is added to the serum. This control is essential and no test is dependable without it. These tubes are tightly covered and kept at body temperature (incubator) for a period of 12 to 16 hours. At the end of that time the dialysates are tested with triketohydrindenhydrat. Take 10 cc. of the dialysate and place in a test tube. To it add .2 cc. of a 1-per-cent solution of triketohydrindenhydrat. Boil continuously for one minute. If positive, an intense violet color appears during the boiling and remains. The amount of color is a relatively quantitative indication of the degree of positivity. Boil control in same way. Compare color of control and test. No case is to be considered positive unless the test is more positive than control. The color deepens on standing, so that the sharpest difference in degree of color appears after standing for about 15 minutes.

INTERPRETATION OF RESULTS.

The value of the test lies not so much in the positivity of the test as in the relative negativity of the control. Contrary to Abderhalden, in animal work our controls were nearly always faintly positive, even when all mechanical sources of error were removed. This fact is very reasonable, for the blood contains amino groups in easily detachable amounts from digestive processes. Serum drawn at the height of food absorption contains some amino groups that are readily dialyzable, but even on starvation some serums give positive reactions after dialyzation. Further, in the case of pregnancy blood may be drawn just at the time of intracorporeal digestion of chorionic villi from the patient's own placenta. In this case the digestion would continue extracorporeally, and give rise to a positive test. Quite obviously the two thimbles used in dialyzing must be of relatively equal thickness, for a thin control tube would be readily penetrated by the small amount of contained amino groups, while in the presence of pregnancy even, the test, if made in a much thicker thimble, would with difficulty be penetrated and would give rise to questionable results. A test, to be positive, must, after removal of mechanical sources of error, give a more pronounced coloration than the control. The control may be negative, then any violent coloration in the test is an indication of pregnancy. Equal coloration of control and test is a negative indication. In our hands tests were made in the above manner, using as control not only the serum without placenta but placenta in distilled water, and in using the desiccated placenta the coagulated placenta of Abderhalden was used as a further control. Results are based only on laboratory experiments. These yielded about 95 per cent of accurate tests. These we were able to follow up by autopsy and exhibition of the fœtus or the demonstration of non-pregnancy. Errors of technique account in the main for the remaining 5 per cent. However, in the presence of unquestionable pregnancy, negative tests have occurred in two experiments. The paucity of data from clinical work does not allow any records being included in the present paper. What we have, however, would suggest that the test is more accurate for human subjects than for animals, in that the controls are many times negative or only faintly positive in women, while in laboratory animals the blood serum normally dialyzes to the extent of giving positive findings in the controls. The decision between positive and negative tests is never doubtful in the human.

These observations are based on 240 tests under varied conditions and at different stages of pregnancy. Our findings lead us to conclude that the sero-diagnosis of pregnancy is both reliable and practical. The method has its limitations, and the technique requires great care. In the hands of the careful worker the method is of sufficient merit to prove of great value to the obstetrician and gynecologist.

LITERATURE.

1. Abderhalden. *Deutsche med. Wochnschr.*, 1912, xlvi, 2160
2. Rose. *Pogg,* 1833, 28, 132.
3. Ruhemann. *J. Chem. Soc.*, Lond., 1910, ii, 2025.
4. Perkin. *Idem,* 1894, i, 480.

71696

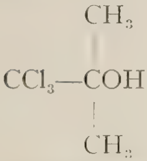
TRIBROMO-*tert*-BUTYL ALCOHOL, $C_4H_7OBr_3$.[1]

BY T. B. ALDRICH.

(From the Department of Experimental Medicine, Parke, Davis & Co., Detroit, Mich.)

In 1881 C. Willgerodt[2] in studying the action of caustic alkalies on a mixture of chloroform in acetone obtained a crystallin compound which resembled camphor somewhat in odor and taste and consequently excited considerable interest.

By analysis and vapor density determinations it was shown that it is an addition product of acetone and chloroform, having the formula $C_4H_7OCl_3$ or $CHCl_3.(CH_3)_2CO$. It was given the name acetone chloroform and later[3] the structural formula



$$\begin{array}{l} \quad\;\; CH_3 \\ \quad\;\;\; | \\ CCl_3\text{—}COH \\ \quad\;\;\; | \\ \quad\;\; CH_3 \end{array}$$

showing it to be trichloro-*tert*-butyl alcohol or the trichloride of acetonic acid. The melting point of trichloro-*tert*-butyl alcohol, which has received the commercial name "chloretone," varies according to the amount of water associated with it. The product recrystallized from solvents containing water and allowed to dry in the open air has a melting point near 80°. The anhydrous compound which Cameron and Holly[4] claim has probably never been obtained should, according to them, melt near but above 97°. Willgerodt[5] states that the acetone chloroform described by him, when recrystallized from dilute alcohol and ether, melted at 80-81° and contained exactly 0.5 molecule of water; whereas the product crystallized from carbon disulfide or repeatedly sublimed is anhydrous and melts at 96°.

The following are some of the properties of chloretone. Boiling point 167° (uncorrected), very volatile in the air, readily volatilized with steam. It has a camphorous odor and taste. It is sparingly soluble in water (0.5 per cent); extremely soluble in ether, alcohol, glacial acetic acid, acetone and chloroform. Soluble also in white neutral oil, and vegetable oils and fats. It is widely used as a therapeutic agent.[6]

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[1] "Brometone" is the commercial name.
[2] *Ber.*, 14, 2451 (1881).
[3] *Ibid.*, 15, 2305 (1882).
[4] *J. Phys. Chem.*, 2, 322 (1898).
[5] *Ber.*, 16, 1585 (1883).

I have mentioned these facts relative to chloretone, its manner of formation, etc., in order to compare it with the analogous compound tribromo-*tert*-butyl alcohol, or "brometone."

This compound is prepared in a similar manner to chloretone, that is, by the action of solid caustic alkalies on a mixture of acetone and bromoform; but it is more difficult to isolate in a pure form. In making chloretone any excess of chloroform can be readily blown off or distilled off. This can be done, but not so readily, with an excess of bromoform and not without considerable loss of the product desired. It can be obtained, however, as a beautiful white crystallin compound, by removing the excess of bromoform, distilling with steam and recrystallizing from a suitable solvent.

It melts at 167-176°, volatilizes in the air but much more slowly than the corresponding compound. An attempt to distil it at ordinary pressure caused decomposition and the evolution of a very irritating gas. No attempt was made to distil it under reduced pressure. It has a camphorous odor and taste. Its solubilities are very similar to those of chloretone. There is no doubt that we have here tribromo-*tert*-butyl alcohol, or the tribromide of acetonic acid, shown by the following analyses which were carried out with a product crystallized from dilute alcohol a number of times and dried in a desiccator over calcium chloride.

	Calculated for	Found.				
	$C_4H_7CBr_3$.	1.	2.	3.	4.	Average.
C	15.43	15.31	15.24	16.13	15.26	15.48
H	2.25	2.37	2.26	2.33	2.41	2.34
O	5.15	....	....	....	....	....
Br	77.17	76.24	75.44	76.84	76.24	76.19

The following analyses were also made from another product dried over calcium chloride, the last two after long drying:

	Calculated for	Found.						
	$C_4H_7CBr_3$.	1.	2.	3.	4.	Average.	6.	7.
C	15.43	15.21	14.58	....	....	14.90	15.7	15.37
H	2.25	2.19	2.18	....	....	2.19	2.32	2.31
Br	77.17	76.92	76.26	76.58	77.1	76.72	77.06	77.14

The above analyses and the difficulty of obtaining very closely concordant results would indicate that there is a varying amount of water present in brometone, this being indicated by the carbon and bromine content, which are low, and the hydrogen content, which in general is high.

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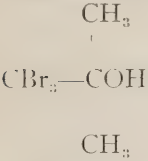
[6] Houghton and Aldrich, *J. Am. Med. Assoc.*, Sept. 23, 1899, p. 77; also *Am. J. Pharm.* (Proceedings), 3, 26 (1900).

The analyses are, however, sufficiently near to assign to it the formula $C_4H_7OBr_3$ and it has no doubt the structural formula



$$CBr_3-\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{COH}}$$

similar to that assigned to chloretone by Willgerodt.

Several attempts have been made to obtain acetonic acid from this product as Willgerodt did from chloretone, but although a small amount of a supposedly organic acid was obtained and an attempt made to convert it into the barium salt, the amount was so small that the results obtained were not sufficiently close to demonstrate its formation, although it was probable. What has been said relative to chloretone (Cameron and Holly) is no doubt true of brometone, *viz.,* that the substance contains water, and in varying amounts, that the water cannot be detected or eliminated by mechanical means, that it is not present to form a hydrate. Therefore it is present to form a solid solution. Brometone is used as a therapeutic agent. For its pharmacological action see Houghton and Aldrich.[1]

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[1] *Am. J. Physiol.* (proceedings), 8, 18 (1903).

71604

ON FEEDING YOUNG WHITE RATS THE POSTERIOR AND THE ANTERIOR PARTS OF THE PITUITARY GLAND.[1]

BY T. B. ALDRICH.

It has been assumed, and certain observations seem to warrant the assumption, that the pituitary gland, either through its own secretions or through the united influence of its secretions with some interrelated gland secretion, has an influence on the growth and nutrition of the body. Whether this growth is due to an increased or decreased activity of the gland has still to be decided. Marie[2] took the former view, while Wood-Hutchinson[3] took the latter, even going so far as to attribute this influence (hormonic) to the anterior lobe alone.

The author, having been interested in the pituitary body for a number of years (especially in the pressor body contained in the posterior lobe), recently carried out some feeding experiments with the anterior lobe, employing young pups for this purpose.[4] The results of these feeding experiments did not show any very marked influence on the growth of the pups collectively, as indicated by their increase in weight, although the growth of certain individuals appeared to be stimulated by the addition of this portion of the gland.

Professor Schäfer[5] claims "that the addition of small amounts of pituitary substances (anterior) to the food of young animals appears to favor their growth; at least it does not impede or restrict it." On the other hand, Cushing[6] states that repeated injections of the extracts of the anterior lobe alone, using the boiled extract or the emulsion of fresh and aseptically removed glands, failed to produce any constant disturbances, except a definite loss of weight.

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[1] Read before the Eighth International Congress of Applied Chemistry, Washington and New York, September 4-13, 1912.

[2] Marle: Revue de médecine, 1885-1886; also Archives de médecine expérimentale, 1891.

[3] Wood-Hutchinson: Transactions of the Pan American Medical Congress, 1894.

[4] Aldrich: this Journal, xxx, p. 320.

[5] Schafer: Proceedings of the Royal Society, 1909, lxxxi, p. 442.

[6] Cushing: Journal of the American Medical Association, 1909, lii, p. 255.

Carletti (1907) injected young animals (intraperitoneally) with a pituitary emulsion (presumably the whole gland), and if his results are to be accepted, they appear to indicate, if anything, a retardation in the growth of the bones, at any rate in the direction of length. Sandri fed young mice with pituitary (apparently with the crude gland to the exclusion of other food), and states that this caused an arrest of the growth, but there were no controls. He also injected young guinea-pigs with an emulsion of the gland, and found in these also the growth diminished.

From the citations given (which include all the observations extant, relative to this subject) it would seem that there is a great diversity of results, which might be attributed to the fact (especially the results of Carletti and Sandri) that the whole gland was employed instead of the separated parts, were it not for the observations of Cushing, who states specifically that he used the separated anterior lobe with the results that a definite loss of weight was observed. Since the writer's previous experiments are not in accord with any of the observations recorded, approaching, however, more closely Schäfer's results, it seemed desirable to repeat his work, and the experiments here recorded were carried out with the view of answering, if possible, the question as to whether either the posterior or anterior lobe of the pituitary gland has any marked influence on the growth of young animals as registered by their weight. In these experiments it seemed desirable to use young white rats instead of pups in order to conform to the conditions obtained by Schafer in his work.

EXPERIMENTAL.

1. *Feeding the Posterior Lobe.*—For this purpose ten healthy white rats, one month old, and all of the same litter, were employed. On February 13 these were separated into two groups of five each (two males and three females in each group), each rat being placed in a separate cage. All were fed the same diet for seven days, that is, until February 20, this consisting of bread and milk (1:2) mixed to rather a thick consistency, and a limited amount of whole corn.

On February 20 the experimental feeding was started, and continued for over three months, each rat being fed daily 30 gm. of the bread and milk mixture (prepared fresh every day), with which was intimately mixed either 50 mg. of desiccated, defatted posterior powder (group A) or the same amount of powdered ovary or testicle alternately (group B). The food was placed in screw-cap jars, with a hole in the cap barely large enough to allow the proper access of the head to the food, but not large enough to allow the food to be scattered around the cage by the feet and tail of the rat. The jars with food were weighed each day before being placed in the cages and again after twenty-four hours of feeding, the proper amount being deducted (this being determined previously from a number of weighings) for evaporation, the difference, with this deducted, being the amount consumed by the rats in twenty-four hours. The jars were thoroughly cleaned each day before refilling, and the cages scalded out every third day, filter paper being placed in the bottom of each. In addition to the feeding already described, each rat of both groups was allowed each day 5 kernels of whole corn. With the exceptions cited all the rats of both groups were treated in every respect the same. The results obtained are given below.

The table on page 440 gives the total weight of the two groups, A and B, together with the average weight of the rats in each group on February 13, when weaned, on February 17, and on February 20, when the addition of posterior lobe or ovary (desiccated) was made, and also their weights with few exceptions, every fourth day thereafter, for a period of over three months. The last column gives the excess of average weight of one group over the other.

The table on page 440 and Fig. 1 show that the weights of the two groups ran along nearly parallel. At no time during the three months did the weights vary by more than 13 gm., and the greater part of the time by only a few grams, 1-6. The average weight variation is still less, 0-3 gm.

The increase in average weight in per cent of original average weight is as follows:

	Posterior.	Controls.
First week (normal diet)...............	33%	35%
April 1	179%	180%
May 20 (whole period)..................	290%	293%

During the feeding the posterior group consumed 7932 gm. of milk and bread mixture (12.3 gm. posterior powder), the

Posterior Group, A (5 rats, 2 males, and 3 females).			Control Group, B (5 rats, 2 males, and 3 females).		
Date.	Total weight.	Average weight.	Total weight.	Average weight.	Excess av. wt. of one group over the other.
Feb.	gm.	gm.	gm.	gm.	
13[1]	212	42	211	42	0
17	243	49	251	50	B[3] 1
20[2]	282	56	286	57	B 1
25	300	60	304	61	B 1
29	338	68	347	69	B 1
Mar. 4	381	76	389	78	B 2
8	421	84	423	85	B 1
12	454	91	462	92	B 1
16	485	97	492	98	B 1
20	512	102	511	102	0
24	530	106	530	106	0
28	575	115	573	115	0
Apr. 1	579	116	586	117	B 1
5	604	121	606	121	0
9	645	129	643	129	0
13	663	133	661	132	A[4] 1
17	686	137	683	137	0
21	706	141	700	140	A 1
25	721	144	717	143	A 1
29	741	148	745	149	B 1
May 3	758	152	767	153	B 1
7	764	153	770	154	B 1
11	784	157	795	159	B 2
15	791	158	804	161	B 3
20	819	164	826	165	B 1

[1] Start of normal feeding.

[2] Start of posterior feeding.

[3] B = Control.

[4] A = Posterior.

controls 7774 gm. (13 gm. desiccated ovary or testicle), or a difference of 158 gm. in favor of the former.

Fig. 1 shows rate of growth of the two groups of rats, posterior and control. Group A, plain line, received the addition of posterior lobe of pituitary to food; group B, dotted line, the controls, received desiccated ovary and testicle. Addition was made February 20. The dates are marked upon the abscissa at proportionate intervals, while the weights are given as ordinates.

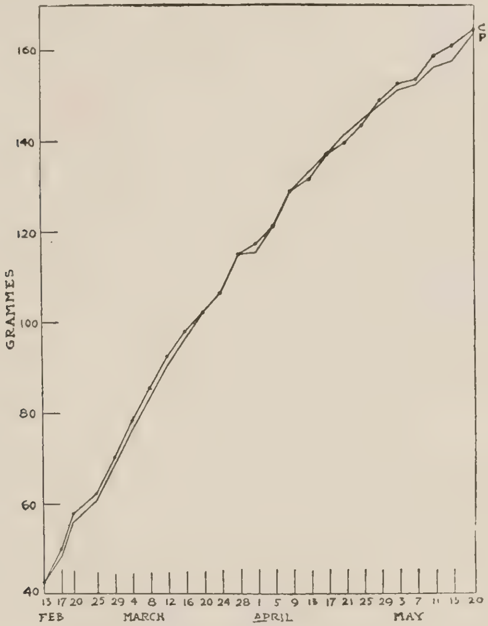

FIGURE 1.—From the table on page 440.

II. *Feeding the Anterior Lobe.*—As in the feeding experiments with the posterior lobe, ten young healthy white rats, one month old, were employed.

On October 23 they were weighed and separated into two groups, each containing five rats (control, four males and one female; anterior fed, three males and two females), each rat being placed in a separate cage. All received the same food for seven days, that is, until October 30, when the experimental feeding was started. The experimental feeding continued for over three months.

ANTERIOR GROUP, A (5 rats, 3 males, and 2 females).			CONTROL GROUP, B (5 rats, 4 males, and 1 female).		
Date.	Total weight.	Average weight.	Total weight.	Average weight.	Excess av. wt. of one group over the other
	gm	gm.	gm.	gm.	
Oct. 23–11	134	27	135	27	0
27	169	34	170	34	0
30 [1]	186	37	189	38	B [2] 1
Nov. 4	207	41	222	44	B 3
8	241	48	263	53	B 5
12	275	55	302	60	B 5
16	294	59	325	65	B 6
20	337	67	370	74	B 7
24	344	69	380	76	B 7
28	366	73	416	83	B 10
Dec. 2	386	77	437	87	B 10
6	395	79	440	88	B 9
10	431	86	477	95	B 9
14	438	88	507	101	B 13
18	452	90	534	107	B 17
22	487	97	556	111	B 14
26	513	103	581	116	B 13
30	499	100	575	115	B 15
Jan. 3–12	532	106	602	120	B 14
8	565	113	650	130	B 17
12	580	116	665	133	B 17
16	600	120	690	138	B 18
20	638	128	731	147	B 19
24	638	128	754	151	B 23
28	621	124	736	147	B 23
31	633	127	758	152	B 25

[1] Feeding started

[2] B = Controls.

The food, with the exception of the substitution of the desiccated non-defatted anterior lobe, was the same as in the previous experiment, and everything was carried out exactly the same. The amount of food consumed was also determined. The results obtained are given in the table on page 442 and in Fig. 2.

The table on page 442 gives the total weight of the two groups, A and B, together with the average weight of the rats in each group on October 23, when weaned, on October 30, when the addition of anterior lobe or ovarian (desiccated) was made, and also their weights nearly every fourth day thereafter for a period of over three months. The last column shows the average increase of the controls over those fed with anterior lobe.

This table (page 442) and Fig. 2 show that although the groups weighed the same at the commencement of the feeding (1 gm. difference), the controls made a steady gain until the end, the control rats then averaging 25 gm. more than the anterior fed.

The inrrease in average weight in per cent of original average weight is as follows:

	Anterior.	Control.
First week (normal diet)	37%	41%
December 10	220%	252%
January 31 (whole period)	370%	463%

During the feeding the anterior-fed group consumed 7472 gm. of bread and milk mixture (12.6 gm. anterior powder), the controls 7771 gm. (13 gm. desiccated ovary or testicle), or a difference of 299 gm. of bread and milk in favor of the latter.

The chart shows the rate of growth of the two groups of rats. Group A, dotted line, received the addition of anterior lobe of pituitary to food; group B, plain line, the controls, received desiccated ovary or testicle. Addition was made October 30. The dates are marked upon the abscissa at proportionate intervals, while the weights are given as ordinates.

CONCLUSIONS.

1. The daily ingestion of 30 mg. of fresh desiccated *posterior lobe* of the pituitary of the ox with the food of young white rats does not apparently stimulate their growth, as shown by their weight. Neither is their growth retarded by such feeding.

2. The daily ingestion of 30 mg. of fresh desiccated *anterior lobe* of the pituitary of the ox with the food of young white rats

certainly does not stimulate; in fact, it seems in these experiments to have impeded their growth.

The poor showing made by the anterior-fed rats may be explained in part by the fact that this group contained only three against four males in the control group, the males being heavier in every instance. This explanation seems plausible in view of my previous feeding experiments with the anterior lobe where young pups were employed. In this case no pronounced difference was noted between the two groups, and the conclusion was drawn that feeding the anterior lobe does not at least impede their growth.

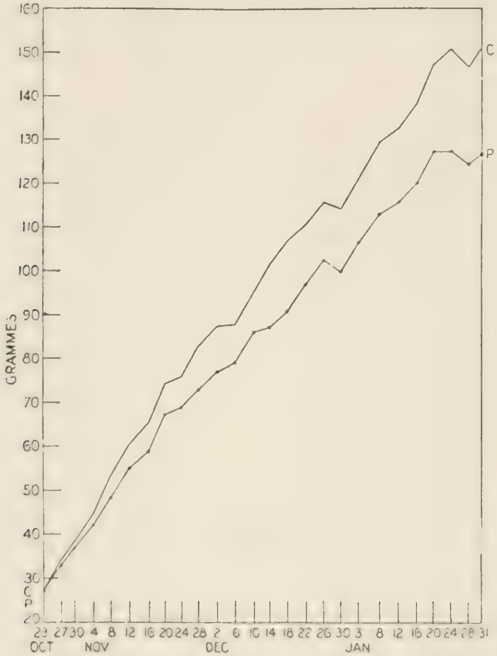

Figure 2. From the table on page 442.

Note.—When the above article was ready for publication my attention was called to an article by Dr. Joseph L. Miller, Chicago, on the "Effect on growth of feeding anterior and posterior lobe of the hypophysis." Dr Miller employed young white rats, and carried out his experiments in much the same manner as the above. His results were completely negative, both as regards weight and skeletal changes, as shown by the *x*-ray. No disturbances were noted, the animals thriving in the same manner as the controls.

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THE RATIONALE OF THE USE OF ADRENALIN IN THE TREATMENT OF ASTHMA.

By Carey P. McCord, M.D., Detroit, Mich.

In the treatment of asthmatics the neurotic and personal elements give occasion to so complex an etiology that prophylaxis is only feasible after a study of the vagaries of the individual patient. In the intervals between attacks so free from symptoms is the patient that the treatment at this period is again essentially prophylactic and embraces the removal of any predisposing conditions, and a general hygienic systematization—all in an effort to ward off the attack, or at least to lengthen the intervals between paroxysms. The forte for drug therapeutics would then appear to be in the relieving of the distress of the attack. The position occupied by adrenalin in this condition is peculiar in that although in the majority of cases it is astonishingly effective, it has attained to but scant use at the hands of the general practitioner. It is upon this value of adrenalin, together with its exemption from any permanent secondary effects, that this paper is based, drawing freely upon results obtained by various clinical and experimental workers.

The immediate pathological conditions exciting the symptoms of the attack have given rise to numerous theories. The most plausible and the one at the present time most substantiated by experimental work is that which ascribes the paroxysm to spasm of the circular muscle coat of the bronchial wall. Secondarily congestive swelling with exudation into the bronchial cavity occurs in many cases, and the spasm of the bronchial wall is probably aggravated by the distention of the distal air sacs pressing upon and further constricting the bronchioles. That such a bronchial spasm may account effectively for the symptom complex produced in asthma has been shown by Brodie and Dixon.[1] They demonstrated that the vagus is the only motor nerve to the bronchial musculature and that in this nerve run dilator and constrictor fibers for the bronchial muscles. Constriction of the bronchioles could be induced by stimulating the constrictor fibers with muscarine, pilocarpine, or electricity, where-upon there occurred all the signs and symptoms characteristic of bronchial asthma. Removal of the constricting influence arrested at once the attack, only to reappear when the constricting influence was re-exerted.

Januschke and Pollak[2] in 1911, after having obtained the experimental bronchial constriction in a manner similar to Brodie and Dixon, demonstrated a dilatation after the intravenous administration of adrenalin. Jackson[3] in 1912 described a series of experimental studies in which the action of adrenalin was investigated. He obtained dilatation of the bronchioles on adrenalin administration, which he concludes is due to stimulation of the bronchodilator fiber endings from the vagus. Jackson indicated further that all his results bear out the opinion that in the intact animal one of the functions of the adrenal glands is to assist by means of their internal secretion in counteracting pathological processes or products, which tend to produce an abnormal broncho constriction. An additional factor in the bronchiole dilatation lies in the observation of Elliott[4] that the administration of adrenalin stimulates a discharge of adrenalin from the adrenal glands, which becomes available for dilatation of the bronchioles. From this group of experimental studies it would appear reasonably well established that adrenalin dilates the bronchial musculature.

The striking clinical results, although explained through many theories, probably owe their beneficial action, at least in part, to this phenomenon. In 1900 Solis-Cohen[5] suggested the use of adrenal extracts in certain forms of asthma associated with vaso motor ataxia on the supposition that asthma was caused by obstruction of the respiratory passages by irregular swelling of the bronchial mucosa similar to angioneurotic edema, and that adrenal extracts by increasing the vascular tone prevented and relieved such attacks.

Lermann,[6] in connection with his employing adrenalin in asthma, has made a study of blood pressure. Before and after the subcutaneous administration of adrenalin, blood pressure measurements were made. He states that the blood pressure which is not high in asthmatic attacks is not increased by hypodermic injections of adrenalin. He attributes the beneficial action of adrenalin to its relaxing the bronchial spasm.

Reilly[7] advocates the use of adrenalin. However, in his cases the asthma was ascribed to nasal pathology and adrenalin was used as a spray upon the contact surfaces with a view to relieving the edematous condition. Eminently satisfactory results were obtained. The inhalation of some adrenalin is a probability in such treatment so that the satisfactory results may have been obtained in a way similar to Segel,[8] who by causing inhalation of adrenalin in oxygen obtained more than transient relief. However, his small number of cases precludes anything more than hopeful possibilities. Equally hopeful results were obtained by Mathews[9] by spraying the nose with adrenalin in acute asthma. Since only a portion of his series had any nasal defect, the spraying of the nose with so much relief lends plausibility to the possibility that inhalation of adrenalin leads to bronchial dilatation.

Freudenthal[10] advocates the endobronchial application of adrenalin for treatment of asthma and cites a series of cases so treated.

Weiss[11] is enthusiastic over results obtained from 3,000 treatments of mild and severe asthmatic cases. His cases obtained permanent relief, and all except ten from a single injection. He supposed that any stimulus contracting the bronchial musculature must follow a nervous condition, or is due to some fault in the composition of the blood, this latter depending on the condition of the blood-forming organs, on the sufficient exchange from the lungs and the skin, on the proper excreting function, and upon the balanced action of the organs of internal secretion. Weiss is of the opinion that through disturbed gland action the circulation is impaired and carbon dioxide accumulates, which in turn brings on the bronchial attack. He used in treatment sterile watery solution of adrenalin in combination with pituitary extract. This he injects subcutaneously. This writer is of the opinion that adrenalin alone will not suffice. No deleterious actions followed the use of the drug in this way. The idea is advanced that since the attack is caused by faulty blood composition the relief following the administration of the drugs is due to the correction of the circulatory disturbance.

Ephriam[12] reported results obtained in his own hands and made a study of results of other workers. Adrenalin treatment, according to this writer, was introduced into Europe by v. Jagic[13] and later was used by Hofbauer.[14] Of his own cases he writes: "I, too, had opportunity to convince myself often that the subcutaneous injection of .75 to 1 c.c. of the marketable 1 per cent. adrenalin solution, almost invariably terminates a severe asthmatic attack." Ephriam further calls attention to Kauert's[15] observation that the site of injection has somewhat to do with the effect of the drug. Injections in the thoracic region react more intensively than those in the more remote portions of the body. Recommendation is made that injections be made in the forearm. The beneficial action of adrenalin given subcutaneously is very transient. In patients who suffer from rare, short attacks, the interval seems in no wise altered even though pronounced relief is given at the time of the paroxysm. In cases of severe protracted *status asthmaticus* the adrenalin effect never lasted more than two hours. However, no ill results were encountered from a large number of injections at two-hour intervals. Ephriam watched the effect of subcutaneous administration of adrenalin with the bronchoscope. Anesthesia of the mucosa was carried out as follows: larnyx with 20 per cent. alypin, trachea and bronchi with 10 per cent. novocain. Such an anesthesia occurs without producing anemia of the bronchial mucosa. With the bronchoscope in place 1 mg. adrenalin was injected into the back and observations were made. In the cases with severe hyperemia an unmistakable paling occurred after 25-60 seconds. From these observations Ephriam concurs in the theory advanced by v. Jagic and Minkowski[16] concerning the production of bloodlessness in the bronchial mucosa upon adrenalin administration. In addition to the spastic process, hyperemia, swelling, and hypersecretion of the bronchioles play important additional causative parts in asthma so that the characteristic ventilation disturbances of the lung result. If, then, as a result of the subcutaneous adrenalin injection, paling of bronchial mucous membranes and extension of the bronchial lumina both occur, not only does normal lung ventilation return, but also the expectoration of the retained secretion becomes possible—all factors of relief. Ephriam, in order to permit the adrenalin to act upon the bronchial mucous membranes more intensively and extensively than is possible from subcutaneous injections, applies the adrenalin endobronchially. This he finds much more effective toward relieving the hyperemia and swelling and the duration is much more lasting, being up to ten hours in cases cited by Möller.[17] Such results follow the bronchoscopic observations which permit only examination of the large bronchi, so that the relaxing action of adrenalin on the bronchioles cannot be studied in this way.

Another point of more than passing interest is to be found in Ephriam's article in connection with the eosinophilia occurring synchronously with the asthmatic attack. In four asthmatic patients treated with adrenalin the blood was examined before and after treatment with reference to eosinophilia. Very regularly the adrenalin administration was followed by a pronounced diminution of the eosinophiles, in part a reduction from 10 per cent. to 3 per cent., but continued study indicated that in these patients the eosinophilia varied greatly. Whether the decrease was due to the adrenalin *per se,* or was concomitant to the changes connected with the asthmatic symptoms, is left in question.

In the above abstracts no attempt is made to cover the entire literature, but rather to cite the experimental basis for adrenalin treatment and attempt to correlate clinical results, explained at present by means of "far-fetched" theories. That adrenalin has found so scant favor at the hands of the general practitioner may be ascribed to the fear of evil secondary effects. Braun[18] warns against the subcutaneous injection of adrenalin, but widespread and extensive clinical experience not only does not bear this out, but indicates that such a fear is groundless. Kirchheim[19] injected daily 12 c.c. of the market solution for many weeks into patients with severe infectious diseases. No secondary effects of evil nature were to be noticed. At the time of injection certain unpleasant results have been pointed out as occurring transiently. Severe palpitation of the heart may occur, which leads to a very pronounced but not accelerated pulse. A peculiar restlessness may set in which may create the patient's alarm, and at times the face may rapidly become very pale, but all these pass rapidly away and at the same time the respiration becomes easier and softer and the rattling and wheezing sounds cease.

From the results indicated above growing out of the use of adrenalin by both laboratory and clinical workers it seems reasonable to conclude:

1. Adrenalin extends the lumina of contracted bronchioles.

2. This dilatation of the bronchioles is probably the basis for the beneficial action of adrenalin in the treatment of asthma.

3. The dilatation follows subcutaneous, intravenous, and endobronchial administrations.

4. The action is transient but very effective in relieving the acute attack. The subcutaneous administration apparently is the most transient.

5. The administration is simple and no injurious results follow as a secondary sequence.

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STANDARDIZATION OF DISINFECTANTS:

SOME SUGGESTED MODIFICATIONS.

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As sources of infection and modes of contagion become more generally known, so also the need of efficient disinfection is more generally appreciated. For many years carbolic acid or phenol proper was the first and almost the only substance thought of as a real germicide, other substances, as copperas and chloride of lime, being commonly recognized as deodorants only. Sophistication of these agents was probably not practiced to any considerable extent.

The advent of proprietary articles for disinfection opened the way for the exploitation of more or less valuable substances of a similar character to phenol but with claims of greatly increased germicidal value. The question naturally arose: Are they more or less valuable than carbolic acid in the recommended dilutions?

So insistent is this question that bacteriologists vie with each other in suggesting methods by which the value can be determined. From the Garnet Method of Krönig and Paul to the latest one to be proposed, that described in *Bulletin 82* of the Hygienic Laboratory, is a long step. This development, however, has been a process of evolution. The methods now employed are modifications of the fundamental principle first published by Rideal and Walker (*Journ. Royal San. Inst.,* 1906) that the value of a disinfectant can best be determined in the laboratory by exposing filtered bacteria suspended in culture medium to the action of diluted disinfectant, the maximum efficient dilutions of sample and of pure phenol being compared to determine the coefficient of the sample, a value which is necessarily relative.

Suggestions have been made by various workers to modify the details in the process. Blythe and others (*Journ. Soc Chem. Ind.,* 1906) suggested the addition of organic matter; Chick (*Journ. of Hyg.,* 1908) that the velocity of the reaction between organism and disinfectant be used as a determining factor; the Lancet Commission (*London Lancet,* 1909), the use of *B. coli communis* in bile salts medium as the organism; Anderson and McClintic (*Bulletin 82,* Hygienic Laboratory), without suggesting any radical change, reviewed the published methods and adopted from them those details which, when slightly modified, seemed to them to meet the requirements for a simple and accurate method. This is a logical course to pursue and since the Hygienic Laboratory Method, as its authors prefer to call it, has been recommended by the Committee of the American Public Health Association for adoption, and has in fact been adopted by some of the State Boards of Health, it seems advisable to suggest at once any changes which will simplify, shorten or improve the process of standardizing disinfectants.

The authors of this paper contributed to the literature on the subject (*Am. Journ. of Public Health,* 1912), a description of the method in use for ten years in the laboratory of Parke, Davis & Co. for standardizing disinfectants. Since the publication of the Hygienic Laboratory Method, both methods have been used as a means of becoming familiar with the details of the latter and of comparing the results obtained by each.

In the course of this work, various modifications have suggested themselves, some of which seem of sufficient value to be published.

Organism. Without any question it is essential that the same strain of the same organism must be used in order to insure uniformity in results. The coefficient obtained with one strain of *B. typhosus* may be 50 per cent. to 100 per cent. higher than that obtained by using another. Even the same strain,—*B. typhosus* (Hopkins), obtained from the Hygienic Laboratory—changed in its relative resistance toward phenol and other coal tar disinfectants to such an extent that coefficients obtained with it as the test organism were 50 per cent. higher than with a fresh culture.

Without special precautions and even in spite of such precautions a culture may change imperceptibly until the results with it are not comparable with the values previously obtained.

Whether the facts above noted are peculiar to the Hopkins culture or to its being grown in the culture medium adopted by the Committee for the Bacteriological Standardization of Disinfectants, this being the one used by Anderson and McClintic, has not yet been determined. A later publication will take up this phase of the problem.

Temperature. Experiments have been conducted which prove that more uniform results may be obtained from day to day by working at a constant temperature. Within reasonable temperature limits the coefficient at different temperatures remains fairly constant because of testing the standard under the same condition. But since the temperature factor is considered of importance by most writers this feature may be adopted; the advantage to be gained, however, is not in accuracy but in uniformity in daily results.

Seeding Tubes. The use of wide seeding tubes recommended by the Lancet Commission and incorporated in the Hygienic Laboratory Method gives greater opportunity for accidental contamination. This, however, may not be serious since if not too frequent the illogical results occasioned by this factor are easily detected and can be ignored in drawing conclusions.

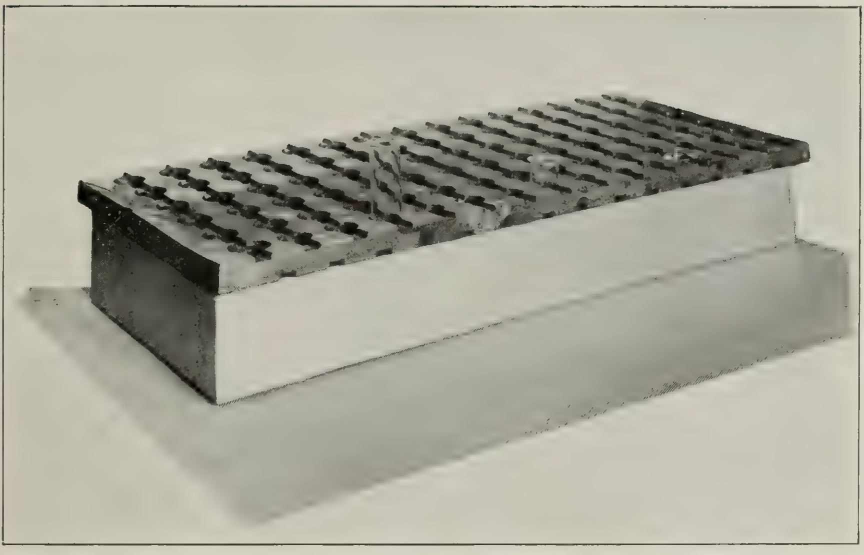


Fig. 1.

Temperature Adjustment. When the wide tubes are used and the temperature is to be adjusted as first recommended by Rideal and Walker a convenient way to carry it out is to have a sheet iron pan three inches deep and of such dimensions that the perforated block suggested in *Bulletin 82,* Hygienic Laboratory, will exactly cover it and rest on its edges (see Fig. 1). Such a pan will hold sufficient water to keep an even temperature for an hour—the time required to test 60 dilutions for a period of 15 minutes each. The block must be modified by being made only seven-eighths of an inch thick and have the holes bored through. When placed on any flat surface, the seeding tubes will stand upright in a convenient position for filling. After filling the measured disinfectant solution into the tubes, the board can be placed over the pan for the succeeding steps of seeding and subculturing. The tubes will pass through the holes to a depth of two inches below the bottom of the board till held by the flange.

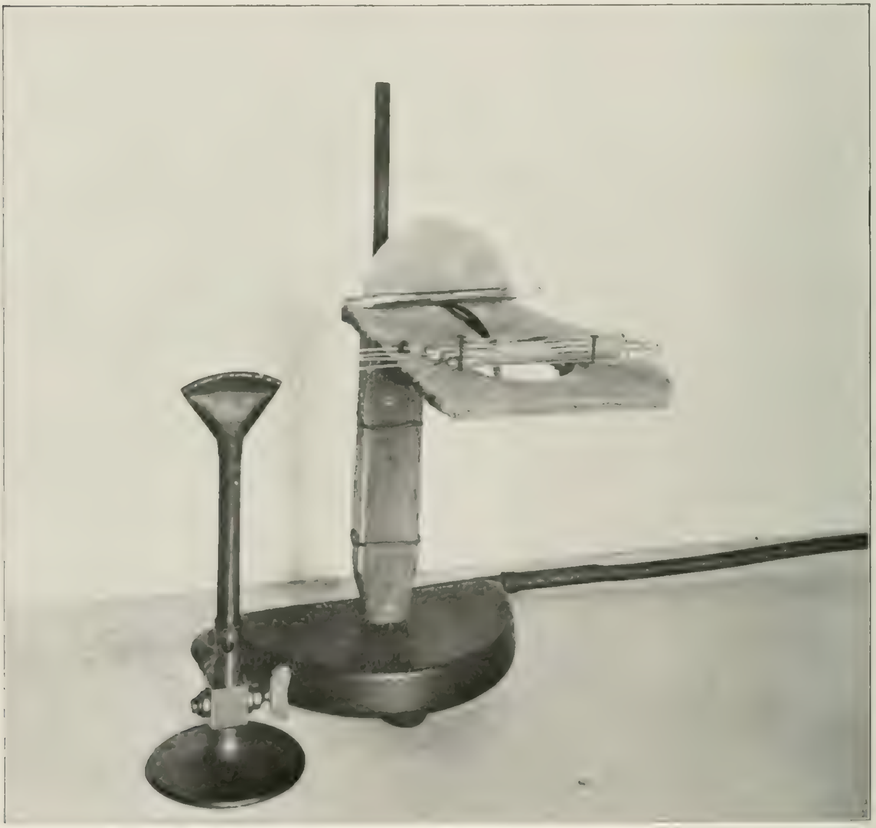


Fig. 2.

Sterilizing Loops. Two simple adaptations of the Lancet Method for sterilizing the platinum loops have been used. One of these (see Fig. 2) consists of a firmly supported slab of asbestos placed in a slightly inclined position. At the higher edge of this slab a disk of sheet iron bent at right angles along its diameter is fastened in such a way that the handle of the loop will lie in the bend in a horizontal position at such a height as to be in the flame of a fish-tail burner. A spring under one side will raise that side slightly above the horizontal to keep the loop over the flame. A slight pressure will depress the disk enough to roll the loop off, after which the disk returns to the position for holding the next loop. The sterilized loop rolls down the slightly inclined slab to an obstruction or to the loop previously sterilized. A cut-out in the asbestos slab under the handles of the loops makes it easier to pick them up. This simple

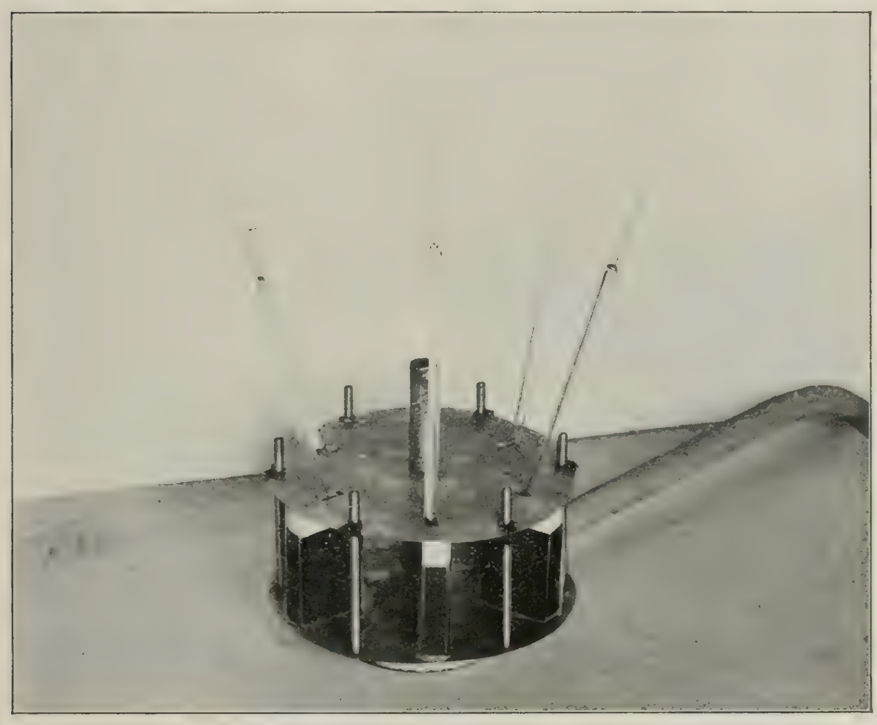


Fig. 3.

apparatus has several points in its favor: 1st, it requires only one small flame for sterilizing; 2d, the wire is never heated more than 15 seconds; 3d, the coolest wire is always nearest to the operator; 4th, only one hand is needed to operate it, since the flame is stationary; 5th, the whole apparatus can be devised from apparatus and material at hand in any laboratory.

The other method illustrated (see Fig. 3) makes use of a revolving holder for the platinum loops. The axis of this holder is the Bunsen burner tube. The handles of the loops are held upright in a slot in which they can be tilted so the loop is either in or out of the flame. Very few movements are necessary to operate this method and it has several advantages over others. One of these is that there is nothing to get out of order; another, that a blue flame is used, thus avoiding the formation of carbon on the wires. This occurs occasionally with a fish-tail burner and is very destructive to the wires.

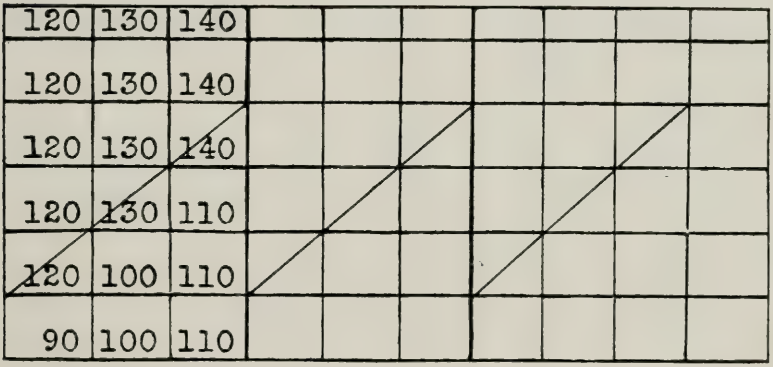
Abridged Methods. When many dilutions are to be tested, especially when one knows approximately what dilutions of the disinfectant will not give growth at 2½ minutes and those that will allow growth after 15 minutes, it is possible to shorten the time of testing and to use fewer tubes of culture medium by discarding the strong dilutions after one or more subcultures have been taken out, and substitute the weaker dilutions which would certainly have given growth if subcultures had been planted after only short exposures.

First. A scheme was outlined to set two rows of ten seeding tubes, each with dilutions of the disinfectants to be tested. If dilutions of phenol and two disinfectants are to be tested, the twenty seeding tubes containing them may be arranged in the board as follows:

Phenol			A			B			
90	100	110	500	550	600	1600	1700	1800	1900
120	130	140	350	400	450	1200	1300	1400	1500
1	2	3	4	5	6	7	8	9	10

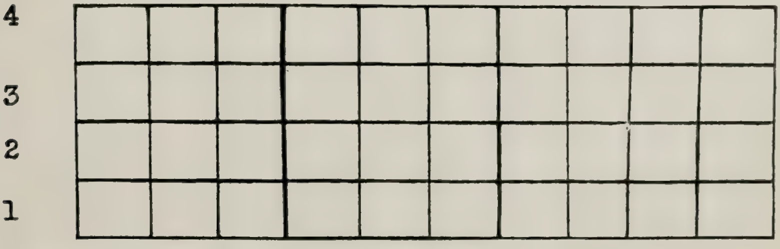
The "90 dilution" is seeded first, and during the 15 seconds allowed for that step the "120 dilution" is also seeded; then the 100 and the 130 dilutions during the second 15-second period. An assistant is needed to shake the tubes before returning them to their proper locations. When all have been seeded in the 2½-minute period, then a subculture is taken from each tube in the lower row. After this, tube 120 is substituted for 90, 500 for 350, and 1600 for 1200. After the 5-minute subcultures have been planted, 130 is substituted for 100, 550 for 400 and 1700 for 1300. After the 7½-minute subcultures have been planted, then 140 is substituted for 110, 600 for 450 and 1800 for 1400. After the 10-minute subcultures have been planted 1900 is substituted for 1500 and no further changes are necessary.

The results can best be recorded by using a diagram representing the rack containing the subcultures, the diagonal line passing through the first subculture of the higher dilution.



120	130	140							
120	130	140							
120	130	140							
120	130	110							
120	100	110							
90	100	110							

Second. This was later further shortened by making subcultures only at 2½ minutes and 15 minutes, according to the following scheme:



4 and 3: 2½ minutes. 2 and 1: 15 minutes.

In the rows 1 and 2 are placed the seeding tubes containing such dilutions of the disinfectants as should show both growth and no growth in 15 minutes. In rows 3 and 4, such dilutions as should show both growth and no growth in 2½ minutes. The rows are seeded in order, 1, then 2, then 3, after which subcultures are taken from 3. Then 4 is seeded and immediately subcultured. Then the first and second rows are subcultured successively.

By this scheme only 20 minutes are required to seed and subculture 40 dilutions and only 40 tubes of medium are needed.

All the data necessary for the coefficient are obtained by this method since the coefficient is based entirely on the greatest dilutions which kill the bacteria in 2½ and 15 minutes. One can choose either a great range in dilutions or have check dilutions, since no time is gained by having fewer than 40 dilutions.

The only objection to be advanced against these abridged methods is that without the results between the 2½ and the 15-minute subcultures, wrong conclusions may be drawn. These, however, are corrected by check tests.

The foregoing points are offered, not as criticisms of any method, but suggestions applicable to methods commonly used. They may be helpful to others as they have been to us in simplifying and shortening an irksome process.

PREVENTIVE MEASURES AGAINST EQUINE INFLUENZA BASED ON ITS BACTERIOLOGY.*

By N. S. Ferry, Michigan.

In equine influenza we are dealing with a disease which is considered to have a relatively low mortality rate, and, therefore, not to be especially feared by the general public, although it is known to be extremely infectious and contagious. In the catarrhal form, Hutyra and Marek give the mortality rate, under favorable conditions, from 0.5 to 4 per cent, while in the pectoral form it ranges from 4 per cent to 16 per cent. Although the death rate is high enough it is not so much the loss due to the dead animals that affects the shippers and owners as it is the enormous expense incidental to the sickness of those that recover. I believe this point has never been fully emphasized. A large percentage of the horses and mules passing through the stock yards and sales stables of the country contract the disease. These animals are incapacitated from a few days to several weeks, and many of them are of little account after recovery, due to the results of complications and sequelæ. The cost of stabling, treatment and care, to say nothing of the loss of time, amounts to millions of dollars yearly, irrespective of the fact that the loss due to diseases among animals is commonly reckoned, by the average observer, according to the mortality rate only. This method of estimation is not absolutely incorrect for the loss due to diseases among animals intended for slaughter, but for others the estimation should be based upon the number of animals affected, and not alone on those that die. In hog cholera, the loss in dollars and cents to the farmer is figured by the number of dead hogs. If the loss due to influenza is computed on the same basis, it is a disease not so much to be dreaded, but if the number of animals affected are included, it is a disease of the greatest economic importance, and yet very few realize the fact. The question then arises, if this is such a contagious disease, if the majority of horses suffer at one time or another with the infection, if 0.5 per cent to 16 per cent die and if many more are more or less incapacitated for life, ought not something to be done to better the conditions, ought not shippers and owners to take better care of their stock, and ought they not, as well as the federal authorities, to make an attempt to prevent the spread of the contagion, or at least to control it by more exact sanitary precautions and hygienic measures? The prevailing idea is that the disease has always been present, that horses have always contracted shipping fever, that those shipped from one part of the country to another must always suffer from acclimation fever; therefore, the disease has always been reckoned with other unknown quantities as profit and loss.

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*Read before the Sixteenth Annual Meeting of the United States Live Stock Sanitary Association, Dec. 3, 4, and 5, 1912.

The diseases or conditions included in this paper are shipping fever, equine distemper, strangles and equine contagious pneumonia, as well as the classical pinkeye. These infections are all contagious, are contracted by horses and mules while in transit from one part of the country to another, are practically found existing side by side in the same stable, under the same stabling conditions and in the same epizootics, and as a rule one attack confers immunity.

If it can be shown that certain pathogenic microörganisms are present in these conditions constantly, and that the majoirty of fatal lesions following these diseases or conditions are due to these certain microörganisms, and that the spread of these microorganisms can be controlled, then it behooves those most concerned to use plenty of common sense, as well as to take advantage of all scientific measures available to stop the spread of the disease and check its ravages. The initial cost of prophylaxis may appear large, but the final saving is enormous. "An ounce of preventive is worth a pound of cure," and in this case it is worth a ton, as compared with the loss incurred if the disease is allowed to continue unchecked.

The primary cause of the disease has never been satisfactorily proved, although we are pretty sure of the organisms that produce the greater number of pathological lesions. Let us turn for a moment to the authorities and see what is said concerning the bacteriology of the conditions recorded in this paper under the head of equine influenza. After summing up the literature to date Hutyra and Marek give us the following conclusions: "The true primary cause of the disease has, up to the present time, not been established. Bacteriological examinations prove only the fact that in the pectoral form (contagious pleuro-pneumonia) two bacteria are principally present, the Streptococcus pyogenes equi, and the Bacillus equisepticus." In strangles the Streptococcus equi of Schűtz is conceded to be the primary etiological factor. As to the relation between the Streptococcus pyogenes equi of influenza, the Streptococcus equi of Schűtz, the Diplococcus of equine contagious pneumonia of Schűtz, and the ordinary Streptococcus pyogenes, it is still a much mooted question. To give some idea as to how the matter stands we will again quote from Hutyra and Marek: "The relation of streptococci from different derivations to each other has not yet been sufficiently established. While Schűtz considers his streptococcus as an independent species, Roth believed it identical with Schűtz's diplococcus of influenza and also the Streptococcus pyogenes of man. Lignières considers the identity of streptococcus of strangles and that of influenza proved beyond a doubt, while he believes that Rosenback's pyogenic streptococcus, which plays a part in purpura hemorrhagica of horses, among other diseases, is an independent species." In fact we might go on almost indefinitely giving the arguments pro and con relative to the relation of these streptococci; but to quote again: "The identity of the streptococci of different origin is still an unsettled question, the solution of which is surrounded by great difficulties, inasmuch as in animal experiments one and the same streptococcus may produce various disease processes (erysipelas, suppuration)."

About two years ago the writer (Studies on the Etiology of Equine Influenza, *The Vet. Jour.*, London, April, 1912) became interested in the question of the etiology of influenza, and from cultures taken at the stock yards in Kansas City, Mo., and from epizootics in Rochester and Pigeon, Mich., by himself, and in East St. Louis and New York City by Dr. R. H. Wilson, the following results were obtained: If cultures were taken from the lower trachea in the very earliest stages of the disease (by going directly into the trachea through the tissues of the neck), a streptococcus could be found in nearly pure culture in the majority of cases, while cultures from normal horses gave no growths, and by taking large quantities of blood aseptically from the jugular vein, a streptococcus was found in 36 cases out of 63 (57 per cent) and in pure cultures in 24 cases (38 per cent). The organisms found by the writer could not be distinguished from the streptococci isolated from strangles, and presumably the streptococcus of Schütz, and it was no doubt the same organism that has been found by so many observers in equine contagious pneumonia, and the condition termed by Hutyra and Marek and other authorities as the "pectoral form of influenza." The organisms found by the writer have been known to pass through porcelain filters, thus showing that they must be extremely minute in some of their life cycle. If this streptococcus has anything to do with the etiology of the disease, the fact that it can at times pass through these filters may account for the findings of those who claim that the etiology is due to a filterable virus. Another interesting fact relative to this organism is that in a certain number of cases the writer was unable to grow it in the ordinary liquid culture media unless a pure culture of Staphylococcus pyogenes albus was planted in the media at the same time, showing the symbiotic relationship between this organism and the staphylococcus. This may also account for the inability of some observers to obtain any growth after blood serum had been filtered, or even before. The writer has had serums that would not give a growth of the organism after repeated trials unless a culture of staphylococcus was planted with it, at which time the streptococcus became very much in evidence. It seems to be characteristic of the organism to grow much more luxuriantly in the presence of the staphylococcus than alone.

As has been said before, the question as to the primary etiology continues to be an open one, but the fact still remains that in all of these conditions we find, from the very earliest stages to the very latest and in all complications, a streptococcus, and if looked for consistently it can be found in every case. Knowing, therefore, that a certain pathogenic microörganism is found in all stages of these varying conditions, and in all complications resulting from the infection, let us turn to the control of this microörganism and see what can be done to stop its spread and to prevent the infection in horses which have never suffered with the disease.

Assume for the sake of argument that the true primary cause of the disease is a filterable virus or some other unknown organism and not this streptococcus. Under these conditions, if we can control this streptococcus and can prevent the complications in a certain number of cases, it must be conceded we have accomplished something, as all authorities are willing to admit that the majority of fatal results in influenza follow complications due to a streptococcus. On the other hand, it is practically agreed by all that the streptococcus of Schütz is the cause of strangles, and as many cannot distinguish between the streptococcus found in influenza, contagious pneumonia, pinkeye, etc., and the species found in strangles, it is not entirely out of all reason to designate this streptococcus as the cause of these other conditions. If this shall ultimately prove to be the case, then by preventing the spread of this microörganism we are controlling the infection to a certain degree, and carrying on a system of prophylaxis that in time is bound to work wonders, and will pave the way to the saving of millions of dollars to shippers and owners yearly.

The methods of prevention are three:

1. Isolation of infected animals followed by well-known approved methods of antisepsis.
2. Practical disinfection and fumigation of stables, stock yards and railroad cars.
3. Prophylactic vaccination before shipment and before exposure.

The first and third methods can be carried out with very little trouble or expense. All it necessitates is a determination on the part of the owner and shipper, and a certain degree of intelligence on the part of those detailed to carry on the work. The second method, however, is not so easy to secure favorable action upon, for it at once imposes a condition on those not directly interested, and, therefore, if anything from that source is ever brought about it would be years before results could be expected. It is not an absolute impossibility, however, to properly disinfect and fumigate all railroad cars in which horses and animals are shipped, and perhaps some day this will be demanded. It is true that influenza may be found on many farms on which the horses are raised and from whence they are shipped, but after all the cars and stock yard stables, reeking with infection, are the most fruitful source of the disease, and yet nothing of a practical nature is being done to protect from infection the animals housed in them. Experience and experiments have shown that the microörganisms under discussion are found in the secretions, excretions and discharges from the infected animals, and, therefore, isolation of the animal, together with careful handling and antiseptic precautions, are the best means available for preventing the spread of the infection.

The most approved method of prophylaxis against any infection of known etiology, however, is that based on scientific experiments, of producing an active immunity in the animal against these microörganisms. According to all authorities, an active immunity may be obtained either by an attack of the disease due to natural exposure, known as naturally acquired immunity, or by deliberate inoculation of the microörganisms into the animal, which is termed artificially acquired immunity. It is the rule that an animal having recovered from influenza and the allied conditions is thereafter immune; therefore, for protective purposes in animals which have never suffered with the disease, we must attempt to produce relatively the same immunity under artificial conditions. An active artificial immunity may be produced by any one of several ways:

(*a*) By injecting into the animal body living virulent bacteria.

(*b*) By injecting living bacteria of diminished virulence.

(*c*) By injecting dead bacteria.

(*d*) By injecting bacterial products secreted or excreted during the life of the bacteria.

(*e*) By injecting bacterial products arising from the disintegration of the cells after death.

For practical purposes it has been found to be the safest procedure in the majority of diseases to use suspensions of dead bacteria. These are termed bacterial vaccines or bacterins. Therefore, in order to render horses immune against the streptococci under discussion they should be injected with vaccines prepared from these organisms. Whether these organisms are the primary cause of the disease is not the main issue. Experience has taught that a large majority of horses can be protected, providing they are properly treated a certain length of time before exposure. Experience, however, is not the only basis for the use of these vaccines. Animal experiments have shown that prophylactic injections of suspensions of these dead organisms will protect against injections of the live virulent bacteria, and tests on the blood of the protected animal, according to the opsonic index, have shown a large increase in the opsonins. All horses, therefore, should be vaccinated before being shipped, and the owner or receiver, in order to protect those horses already in the stables, should give them a thorough course of prophylactic treatment. One injection of a large dose will do for a short interval, but three injections of properly graduated doses with intervals of a few days will give better results, and ought to suffice to protect the horse for at least six months or perhaps a year. As these vaccines have been in general use but a relatively short time, one is not warranted at the present time in laying down a hard and fast rule as to the length of immunity conferred after the prophylactic treatment.

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CORRECTING WATER.

Methods of Treating Hard and Alkaline Waters—How to Remove Objectionable Ingredients—Ways of Improving Water for Washing Purposes and for Diluting Dips and Disinfectants.

By H. C. Hamilton.

(From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.)

Water as supplied to cities for domestic purposes is no longer looked upon as merely water, but as a suspension of bacteria and dirt in a solution of salts. As such, the health officer, the city chemist and bacteriologist, and, in yearly increasing numbers, the consumer, are looking at it with growing suspicion.

It is stored in settling basins or filtered to remove suspended matter, and is treated with germicides if dangerous bacteria are present. In manufacturing plants, it is mixed with substances intended to remove or make less objectionable the ingredients which form boiler scale or which corrode the boiler. But for drinking, cooking, washing and other domestic purposes no attention is usually paid to the substances in solution.

While water for drinking purposes may be very objectionable to one unaccustomed to the presence or absence of certain soluble salts, very few salts found in water other than sea water make it unfit for drinking purposes. In fact the presence of lime and magnesia in reasonable quantities is considered to be distinctly beneficial, and when one is accustomed to a water of this character, "soft" water is very insipid. For washing purposes, however, the presence of any considerable quantity of salts in the water is decidedly objectionable, for if they do not combine and form insoluble compounds with the soap, they may render the soap itself insoluble and destroy its detergent properties. Common salt or sodium chloride does not form any compound with soap, but merely makes it insoluble. Sea water, however, and most natural waters containing this salt, usually have lime and magnesia compounds associated with it, which makes it unfit for washing with ordinary soap without correcting the water.

EMULSIFYING COAL-TAR DIPS.

There is another important use for water for which soft water is just as necessary as for steam and washing purposes, namely, to dilute a product which depends on soap for its solubility. Most of the coal-tar dips and disinfectants, whose principal ingredient is creosote oil from coal tar, are made soluble in or miscible with water by means of soap. When these are mixed with water, the insoluble oils are broken up into minute globules and held in the form of a white emulsion if the soap is readily soluble in the water. If the water is of such a character that the soap does not dissolve or is made insoluble, it is impossible to obtain a proper mixture of the active oils with the water. In such cases there usually results a dark muddy mixture which is either dangerous or unfit to use for dipping animals. If the oil floats on the surface, the first animals dipped are coated with almost pure oil, which in this condition is often very corrosive. If the oil is carried to the bottom with the curdy soap, very little will come in contact with the animal and no beneficial action follows.

It is the purpose of this article to point out the objectionable features of the various foreign substances which occur in the waters of the country, means by which they may be readily identified, and the measures necessary to eliminate them from waters intended for washing purposes, for steam boilers, and for diluting coal-tar dips.

HARD AND SOFT WATERS.

Pure water is an almost unknown substance because water is so good a solvent that it can rarely be found without some foreign substance dissolved in it. Rain water before it reaches the ground has dissolved or suspended in it a large part of the constituents of the dust, smoke and gases continually arising from populous manufacturing communities. This is comparatively small, however, outside the vicinity of smelting, refining and other chemical works, and rain water is usually applicable for almost any purpose without treatment. It is objectionable as a beverage because of its freedom from salts. This characteristic of rain water is particularly desirable for all purposes where soap is to be dissolved in it, and is responsible for the term "soft water."

Hard water is that containing calcium or magnesium salts, particularly the former, since the average hard water contains two to ten times as much lime as magnesia. Temporary hardness is that form in which these bases occur as carbonates and bicarbonates resulting from the passage of the water over and through limestone. A large part of these dissolved salts are precipitated by boiling the water, a fact which is responsible for the term "temporary hardness." Where boiling is not practicable and softening is necessary, it can best be accomplished by the addition of either potash or soda lye or, when neither of these is available or is too expensive, by the use of fresh, water-slaked lime. Whether these salts which are ordinarily insoluble in water are present as bicarbonates or in a colloidal state (see Allen, J. S. C. I. 7, 795-806) is not known, but the commonly accepted theory is that carbon dioxide is present in excess of that necessary to form the carbonates. The basis for this theory is that when carbon dioxide is removed by combination with the lye or by boiling, an immediate precipitation of the carbonate of lime occurs and the water is "softened." This can be illustrated by a chemical formula only on the supposition that lime is present as a bicarbonate as follows:

(1) $Ca(HCO_3)_8+2NaOH+aq.=CaCO_3+NA_2CO_3+aq.$
(bicarb. of lime) (caustic soda) (precipitate)

Or,

(2) $Ca(HCO_3)_2 + Ca(OH)_2 + aq. = 2CaCO_3+4H_2O.$
(bicarb. of lime) (slaked lime) (precipitate)

"Permanent" hardness is distinguished from "temporary" in the fact that boiling does not materially decrease it. It is the name applied to hardness remaining after the water is boiled. It is caused by the presence of the sulphates of lime and magnesia, and is more objectionable than the temporary hardness because these salts are soluble in water to a much greater degree. In fact magnesium sulphate or Epsom salt is soluble to such an extent that where it predominates over the calcium sulphate or gypsum, the water is no longer classed as "hard," but is called "alkaline." Permanent hardness may be recognized by the action of washing soda or sal soda, technically known as "sodium carbonate," which produces a precipitate according to the following equation:

$$(3)\quad \underset{\text{(gypsum)}}{CaSO_4} + \underset{\text{(soda ash)}}{Na_2CO_3} + aq. = \underset{\text{(precipitate)}}{CaCO_3} + Na_2SO_4 + aq.$$

$$(4)\quad \underset{\text{(Epsom salt)}}{MgSO_4} + \underset{\text{(soda ash)}}{Na_2CO_3} + aq. = \underset{\text{(precipitate)}}{MgCO_3} + Na_2SO_4 + aq.$$

ALKALINE WATER.

Water which contains as its principal foreign ingredients sodium or potassium carbonate or bicarbonate is technically known as alkaline water. Other substances, however, are found in water which is classed as alkaline, particularly the sulphates of sodium and magnesium. The deposit left from the evaporation of such waters in hot, dry climates is very irritating to the eyes, nose and throat. It is popularly known as "alkali" and is the name applied to any noticeable white coating on the soil.

In some of the Western States the soil and the water are so impregnated with this "alkali" that it is almost unfit for any purpose. It must even be used with extreme caution for irrigation purposes, since if allowed to evaporate instead of being promptly drained off, the accumulation of alkali in the soil is so excessive as to prevent the growth of plants. Such a water must be treated with a percipitant that will remove all the constituents. The theoretical limit of salts in water for irrigation purposes is 300 parts sodium chloride (common salt) or sodium carbonate (black alkali) and 3000 parts of the less harmful salts per million parts of water.

The Arabs in the Algerian oases of the Sahara Desert, however, are using for irrigation purposes water containing as high as 8000 parts of soluble salts per million parts of water, and in some cases one-half of this, or 4000 parts, is sodium chloride.

The Bureau of Soils has investigated their methods and finds that by a liberal use of the water on well-drained soils, the land can be brought to and kept in a condition suitable for any crop which is adapted to the climate. Two or three years are often required to remove the accumulated salts before attempting cultivation, and quick drainage must be employed to prevent a further accumulation.

In the ordinary "hard" water due to gypsum, the sodium sulphate remaining in solution after treating with washing soda is not objectionable because there is not sufficient to make soap insoluble. When, however, "alkali" water containing a large amount of magnesium sulphate is treated with washing soda, the magnesium is removed, but so large a proportion of sodium sulphate may remain that the emulsifying properties of the water are not greatly improved. In this case, the water can best be treated with fresh water-slaked lime according to the equation:

$$(5) \qquad \underset{\text{(Epsom salt)}}{MgSO_4} + \underset{\text{(water-slaked lime)}}{Ca(OH)_2 + aq.} = \underset{\text{(precipitate)}}{Mg(OH)_2 + CaSO_4} + aq.$$

Both the reaction products are practically insoluble, and if magnesium sulphate were the only foreign substance present, the water after settling should be in a satisfactory condition for most purposes. If, however, the water also contained gypsum, which, as has been noted, is nearly always associated with Epsom salt, an additional treatment with washing soda is necessary, since the application of slaked lime does not affect this ingredient. The reaction in this case is that shown in equation (3). It is necessary to add a small amount of washing soda to the water after the lime treatment in every case because even if no gypsum was present in the water naturally, it is difficult so to adjust the addition of slaked lime as to have sufficient for the combination without an excess which is very objectionable. This excess of lime and the gypsum formed in the reaction are removed by the use of washing soda.

The use of these two precipitants, lime and soda ash, mixed together before applying to the water, has been recommended (*Jour. Soc. Chem. Ind.,* June 30, 1891) in an address by Archbutt and Deely on "The Treatment of Hard Water." A similar mixture is that used by the Chicago, Milwaukee and St. Paul R. R. (*Chemical Engineering,* Stillman, page 97), two parts caustic soda and one part washing soda mixed and dissolved in a gallon of water.

These two methods are both applicable for the treatment of feed water for steam engines, but for dissolving soaps better results are obtained by the method suggested above, namely, to follow the treatment with water-slaked lime by an addition of soda ash or washing soda.

"Black alkali" waters are those containing sodium carbonate and sulphate in solution. If no calcium or magnesium salts are present, no corrective treatment is needed unless there is an excessive quantity, but usually some of these are present and an addition of either lime or soda is necessary.

Acid waters, rendered so by leaching decomposed organic matter or by receiving the drainage from mines or factories, and water containing the chlorides of calcium and magnesium and the soluble salts of iron and aluminum, are to be corrected only by use of soda. Hydrogen sulphide occurring in some spring or mine waters, while not greatly affecting a soap solution, is objectionable in many cases and may be removed by ferrous sulphate (copperas).

TESTING AND CORRECTING WATERS.

From the foregoing it is evident that a chemical examination of a sample of water would supply valuable information as to the best method of correcting it when it fails to emulsify a coal-tar dip, forms a curd instead of a foamy lather with soap, or forms scale in the boiler. However, some simple tests may be devised to determine what treatment to apply.

If an emulsive coal-tar product will not mix with a certain water, proceed as follows:

1. In 1 pint of the water dissolve 15 grains of sodium carbonate. If a considerable cloudiness occurs, shake thoroughly, and after one hour pour off the water from this sediment and test its emulsifying properties, comparing with the untreated water.

2. If the emulsion is not good or if only a slight cloudiness results from the first test, use a second pint sample of the water and add 5 grains of lye. If cloudiness forms, stir thoroughly and allow to stand 1 hour. Pour off and test the water standing above the precipitate if precipitation has occurred.

3. If the emulsion is not good, although a precipitate has formed, take another pint sample of the water and add 15 grains of sodium carbonate and 5 grains of lye. Shake or stir the water thoroughly and test the sample, one hour after mixing, by noting the quality of the emulsion it will make.

In every case the lye or soda is to be dissolved in some water before adding to the sample.

If the emulsion is still unsatisfactory, it is probably due to ingredients of special character and requires a chemical examination before further directions are possible. If one of the three methods is found to improve the emulsion, the next step is to determine the amount of necessary ingredients to be used per gallon.

If, in preliminary test (1) a precipitate occurs, the necessary amount of sodium carbonate can be roughly determined by the following procedure:

(*a*) Measure out 1 gallon of water very carefully into a container that will hold 2 gallons.

(*b*) Prepare a test solution by dissolving 15 grains of sodium carbonate in 1 ounce of water. This will be known as Test Solution No. 1. There should be three or four lots of this solution prepared beforehand.

(*c*) Add one lot of Test Solution No. 1 to the gallon of water and stir or shake thoroughly.

(*d*) Take out some of the water in a glass tumbler or bottle and add to it a second lot of Test Solution No. 1. Watch closely to see if a cloudiness occurs when the test solution is slowly dropped in.

(*e*) If this occurs, mix the tumblerful with the gallon lot and again stir or shake thoroughly. Repeat (*d*) and (*e*) until finally no cloudiness occurs on adding Test Solution No. 1 to a portion of the gallon sample.

If in the preliminary test (1) no cloudiness occurs, but forms on applying test (2), the necessary quantity of lye or lime can be determined as follows:

(*a*) Measure out 1 gallon of the water as before into a 2-gallon container.

(*b*) Prepare a test solution by dissolving 5 grains lye in a tablespoonful of the water. This will be known as Test Solution No. 2. There should be three or four lots of this prepared beforehand.

(*c*) Add one lot of Test Solution No. 2 to the gallon sample and shake or stir thoroughly.

(*d*) Take out some of the water in a glass tumbler or bottle and add to it a second lot of Test Solution No. 2. Watch closely for a flocculent precipitate to form when the test solution is dropped in.

(*e*) If this occurs, mix the tumblerful of water with the gallon lot and stir or shake thoroughly.

(*f*) Repeat (*d*) and (*e*) until no further precipitation occurs on adding Test Solution No. 2.

If in the preliminary test (3) the water was found to require treatment with both lye and sodium carbonate, proceed as follows:

(*a*) Use either of the two 1-gallon samples corrected as above and apply the other treatment, following the directions the same as for a fresh sample of water. If it is found after adding several lots of the test solution that the water is too cloudy to determine whether any further cloudiness occurs on adding another lot of either test solution, allow the water to stand for a short time and this condition will disappear.

From the data obtained for one gallon of water, as above, the amount of lye, lime or soda ash necessary for 500 gallons of water may be calculated as follows:

A. If lye is to be used. If 5 grains of lye (one lot of Test Solution No. 2) was sufficient to correct one gallon of water, use one-half pound of lye for each 500 gallons. Dissolve the lye in water, using one quart for each half-pound of lye, and, when completely dissolved, mix thoroughly with the water in the vat.

Example: If three lots of Test Solution No. 2 were used for one gallon and the vat holds 1000 gallons, there will be required 3 pounds of lye, which should be dissolved in 6 quarts of water.

B. If lime is used instead of lye. Exactly the same calculation applies in this case as in A to determine the necessary amount. Use only fresh stone lime. Slake it in water, using about a gallon of water to a pound of lime. When slaked, stir in enough water to make a thin milk and add this to the water in the vat.

C. If soda ash is to be used. For each lot of Test Solution No. 1 (15 grains of sodium carbonate) used to correct one gallon of water, use 1 and 1/5 pounds soda ash for each 500 gallons. Dissolve the soda ash in water, using 5 pints for each pound.

When completely dissolved, stir the solution thoroughly into the water in the vat.

Example: If three lots of Test Solution No. 1 were used to correct one gallon of water, use 5½ pounds for each 500 gallons. If the vat holds 1000 gallons, use 11 pounds of soda ash dissolved in 6½ gallons of water.

D. If sal soda or washing soda is to be used. For each lot of Test Solution No. 1 (15 grains sodium carbonate) used to correct one gallon of water, use 5 pounds of sal soda or washing soda to correct each 500 gallons of water.

Example: If three lots of Test Solution No. 1 were used in one gallon of water, use 15 pounds sal soda in 500 gallons or 30 pounds for 1000 gallons. Dissolve the soda in a half gallon of water for each 3 pounds and stir the solution thoroughly into the water in the vat.

E. If both lime and soda ash are necessary for correcting the water, always use the lime treatment first. Any excess of lime will be removed by the subsequent addition of soda ash. See *B* and *D* for quantities.

When the water has been treated by one or more of the methods described above, the resulting precipitates usually settle to the bottom in a few hours. A floating scum can be either skimmed off or, if stirred slightly, it will settle. This sediment is more or less objectionable, therefore, if it is possible to draw off or dip the water into another container, leaving the sediment in the first tank, a better emulsion will result when coal-tar products are mixed with the water.

A better emulsion is obtained if the preparation is thoroughly mixed with an equal amount of water before diluting it to the full amount.

For example: If 7 gallons of dip are to be added to 500 gallons of water, it can be emptied into the vat after 7 or 8 gallons of water have been put in, or it can be mixed with 7 gallons of water and then poured into the remainder of the 500 gallons which is already in the vat. This suggestion applies whether one uses rain water or softened water.

When it is impossible or inconvenient to obtain the chemicals referred to above, there is another method of examining and correcting a hard or alkaline water.

F. For correcting the water with soap.

(*a*) Fill a quart bottle exactly half full with the water.

(*b*) Make up several lots of Test Solution No. 3, which is a solution of soap 20 grains in a tablespoonful of soft water (either rain water or water previously softened by addition of lye).

(*c*) Add one lot of Test Solution No. 3 to the pint of water; shake thoroughly and note whether a foamy lather results which is permanent for five minutes. If the water is very hard or alkaline a curdy lather is formed which is confusing. To remove this, filter or strain the water through a wet piece of cotton flannel cloth, or canvas, and shake again to note whether a permanent foamy lather is formed.

(*d*) If not, repeat (*c*) in every particular until such a lather results as is described in (*c*).

To correct each 500 gallons of water will require 12 pounds of soap for each lot of Test Solution No. 3 that was used for one pint of water. The soap should be dissolved by boiling in one gallon of soft water to each pound of soap, and the solution of the soap very thoroughly stirred into the water. More stirring is needed to mix the soap thoroughly with the water than for the other chemicals. The curdy mass floating on the surface of the water after the above treatment can be largely removed by skimming. This should be done before adding the dip to the water. If rain water is not available, the water in which the soap is dissolved should have added to it sufficient lye and soda to soften it. For this purpose add about 30 grains of lye and 60 grains soda ash to each gallon of water. This procedure can be omitted, but a much better solution of the soap results if it is possible to soften the water as suggested.

The foregoing description of methods for correcting hard or alkaline water is written on the supposition that no accurate chemical analysis of the water has been made. If an analysis of the water is available, more specific directions can be given without making the tests described above. In this case proceed as follows:

For each grain per gallon of calcium carbonate present, use ½ grain of lime or 1/3 grain of lye or 8 grains of soap per gallon.

For each grain per gallon of magnesium carbonate, use 2/3 grain of lime or ½ grain of lye or 8 grains of soap per gallon.

For each grain per gallon of calcium and magnesium sulphates use 1½ grains of soda ash or 4 grains of crystallized sal soda or 6 grains of soap per gallon.

If magnesium sulphate (Epsom salt) and sodium sulphate (Glauber salt) are present in an alkali water to exceed 60 grains per gallon use 2/3 grain of lime or lye per gallon for each grain of these salts present. For smaller quantities use sal soda or soda ash or soap as previously directed.

Note: If the analysis is given in parts per million divide by 17 to reduce to grains per U. S. gallon.

In every case where lime is used for correcting a water, follow it with the addition of 1/5 grain of soda ash or ½ grain sal soda for each grain of lime used, in order to remove any soluble lime compounds remaining in the water.

When it is impracticable or inconvenient to apply the tests suggested in the foregoing directions, almost any water supply can be corrected by the following treatment: For each 500 gallons of water to be used, dissolve 2 pounds each of lye and soda ash (or 5 pounds sal soda) in 2 gallons of hot water, and stir this solution thoroughly with the water in the vat. Allow at least 1 hour for the chemicals to act before adding the dip. To insure a proper mixture of the dip it should be mixed with an equal quantity of the water before being poured into the vat. The reason for this is, that if the undiluted dip is poured into a large quantity of water, there is a tendency for it to carry to the bottom. It is then difficult to obtain a satisfactory solution unless there are exceptionally good facilities for stirring the entire contents of the vat.

Almost every ingredient commonly found in water can be removed by one of the methods described above. Waters which have had their foreign ingredients removed by the foregoing treatments are corrected for practically every purpose, since the methods described are designed to eliminate these substances as completely as the best "boiler compound" will do it. No more efficient substances than those described are available for removing the scale-forming ingredients of water or to improve it for washing purposes, and for diluting dips and disinfectants.

DURATION OF IMMUNITY FOLLOWING SMALL-POX VACCINATION.*

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(From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.)

A certain degree of reticence is felt about recording observations on the subject on which so much work has been done and with which I have had a relatively short experience. The collection of the present data, however, was prompted, first, by the observation made in a larger series of cases that successful revaccination after relatively short intervals is met with surprising frequency, and, second, the variance of my results with the ideas commonly held by physicians with whom I have come in contact. In dealing with patients reporting for compulsory vaccination, and who have a certain degree of antipathy to being inoculated, not infrequently objections are offered based on statements alleged to have been made by physicians by whom they have been previously vaccinated. If the patient has an unusually sore arm not only he, but sometimes his physician as well, is inclined to believe that a permanent protection has been conferred.

I was directly prompted to report my observations by an editorial appearing in *American Medicine,* in February of this year, and from which the following quotation is made: "It is almost a rule for revaccination to be unsuccessful, and there are no cases of small-pox on record in which there is a history of proper vaccination and revaccination which have undoubtedly been successful." A little further on the editor refers to certain statistics published by Dr. Kitasato of the Imperial Institute of Tokio and said: "He estimated that immunity was practically gone ten years after vaccination. These figures cannot be accepted for a moment. The error was doubtless due to mistaking an inflammation from pus organisms as an evidence of successful vaccination. The British Royal Commission states that immunity generally lasts for nine or ten years, but it is justifiable to conclude that the evidence on which this opinion was based was also defective from the inclusion of spurious cases. Proper vaccination is far more effective than this, and we revaccinate entirely too often." From these quotations it is evident that the editorial writer believes immunity from vaccination to be practically permanent in most cases.

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*Read in the Section on Preventive Medicine and Public Health of the American Medical Association, at the Sixty-Fourth Annual Session held at Minneapolis, June, 1913.

Kitasato's results, which were the object of this criticism, were based on a series of 931 revaccinations. He obtained successful revaccinations approximately as follows:

After 1 year	14 per cent.	After 6 years	64 per cent.
After 2 years	33 per cent.	After 7 years	73 per cent.
After 3 years	47 per cent.	After 8 years	80 per cent.
After 4 years	57 per cent.	After 9 years	85 per cent.
After 5 years	51 per cent.	After 10 years	89 per cent.

Millard[1] states that the government report of the German Confederacy shows from 91 per cent. to 93 per cent. successful revaccinations after ten years from the primary, and concludes with the statement that "immunity acquired through vaccination begins to disappear from the second year, and by the tenth year it disappears almost completely."

These different opinions are mentioned simply as illustrating the decided variance of ideas in regard to this question. It would seem as though the subject were worth more than a passing consideration, both because of its relation to public health and because of its bearing on the antivaccination movement. It can scarcely be questioned that systematic revaccination is almost as important as the initial inoculation, and that modern methods of small-pox prevention must take this into account. A system of infantile vaccination which is not followed up by revaccination is only a half measure, and half measures often do a great deal of harm, because they create a false sense of security. Furthermore, if a vaccinated person, whose immunity has lapsed, contracts the disease, it creates a bad effect on the community in which he lives, a distrust in the efficacy of vaccination as a preventive measure.

The literature shows a surprising dearth of data on duration of immunity, presumably due to the difficulty of obtaining definite histories on cases reporting for revaccination, and to the fact that revaccination is not carried out in a systematic way. As a general rule, revaccination, and in a great many sections it is unfortunately true of primary vaccinations as well, is performed only in the event of a small-pox scare and under circumstances which are not favorable to the collection of statistics.

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[1] Millard: *Bost. Med. Journal*, March 22, 1913.

In the present series we had a total of over five hundred cases, sixty-six of these being primary. In only two hundred and fifty-one of the secondaries were we able to get a history as to previous vaccination. It may be mentioned that in considering the periods since previous vaccination no recognition was given to negative inoculations, the history being taken from the last successful vaccination.

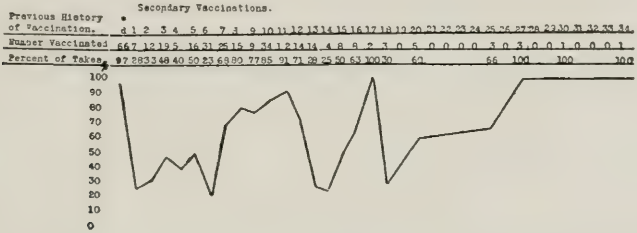

Fig. 1.—Chart showing results of secondary vaccinations. Total number vaccinated, 317. Primary cases vaccinated, 66. Secondary cases vaccinated, 251. Per cent. of takes in primary cases, 97. Per cent. of takes in secondary cases, 58. Star (Footnote *) indicates primary vaccinations. Following figures indicate lapse of years since a successful vaccination.

RESULTS OF REVACCINATION OBTAINED IN THESE CASES WERE AS FOLLOWS:

INTERVAL IN YEARS SINCE LAST VACCINATION	NUMBER VACCINATED	PERCENTAGE OF "TAKES"
1	7	28
2	12	33
3	19	48
4	5	40
5	16	50
6	31	23
7	25	68
8	15	80
9	9	77
10	34	85
11	12	91
12	14	71
13	14	28
14	4	25
15	8	50
16	8	63
17	2	100
18	3	33
20	5	60
25	3	66
27	3	100
30	1	100
34	1	100

With the exception of the patients vaccinated after the first year our results accord fairly well with those reported by Kitasato.

It is highly improbable that in the larger series of cases we would have as high as 28 per cent. "takes" in patients who had been successfully vaccinated the year previous. This has been regarded as being more or less of a coincidence, but the fact that revaccination is frequently successful after intervals of from one to three years is in itself significant, and indicates the advisability of the physician being somewhat guarded in statements made as to the duration of immunity, and points to the importance of systematic revaccination.

The question arises as to whether some of the cases included in this series and classed as successful vaccinations might have been spurious ones, as has been suggested in the editorial above referred to, pyogenic infections being mistaken for successful inoculations. This hardly seems probable as there is usually no great amount of difficulty in differentiating between a pyogenic infection and a vaccine pustule, and I believe that I have been reasonably careful in drawing conclusions.

It must be freely admitted that the successful vaccination of these patients is by no means evidence that they would have been susceptible to natural small-pox. It is reasonable to suppose that a higher degree of immunity would be required to resist the direct inoculation than would be necessary to protect the patient against infection in the usual way. Furthermore, clinical experience has demonstrated that systematic revaccination even at intervals of ten years is very efficient in protecting a country or community against small-pox. In Germany revaccination is carried out in the eleventh year, while small-pox is very rare; the same is true of Japan, and it is a universal experience that small-pox in individuals who have been successfully vaccinated within five or six years is decidedly unusual. In this connection, King reports, in the *Lancet* of May 17, 1913, some interesting data based on observations made on Asiatics (Shans, Burmese and Chinese). In a group of 380 adults who had been vaccinated when under 15 years of age 5.6 per cent., and in a group of ninety-four who had been inoculated above that age, 3.2 per cent., had been attacked by small pox in later life. In a series of 312 patients who had been inoculated under fifteen years, vaccination resulted successfully in 80.3 per cent, and in twenty-two with a history of having been previously vaccinated after their fifteenth year, 81.8 per cent. resulted in "takes." In ninety-six adults, who had suffered from natural smallpox at various ages, and who were freely marked, 75 per cent. were successfully vaccinated.

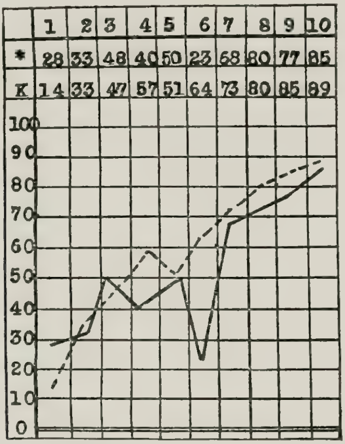

Fig. 2.—Comparison with Kitasato's results. The results in the present series are indicated by solid line and starred figures. Kitasato's results are indicated by dotted line and figures preceded by K.

It can scarcely be doubted that our present system of vaccination is not sufficiently rigid, and that a single successful vaccination confers a false sense of security, not only on the patient, but in many instances on the physician as well. The disease has been widely disseminated during the last year or so, and the indications are that the country as a whole is far from being well protected. In view of the general apathy of the medical profession toward vaccination this is not especially surprising. While the great bulk of medical practitioners undoubtedly believe in vaccination, it is in many instances afforded only a half-hearted support, and is rarely urged by the family physician, excepting in the presence of an epidemic of the disease, or in the case of direct exposure. Even in large cities one is surprised to learn of the large number of people who had never been vaccinated, and in small towns and country sections vaccination is even more neglected. The majority of those from rural districts who have reported to me have never been vaccinated, or even been approached on the subject. These phases of the question, of course, have nothing to do with the present subject, and are merely mentioned in passing as being, with the transient immunity, factors which contribute to the prevalence of small-pox in the United States.

ABSTRACT OF DISCUSSION.

Dr. C. N. Hensel, St. Paul, Minn.: I have examined about a thousand cases of small-pox in my work in the St. Paul Health Department. Of this number, but four patients had been previously successfully vaccinated and showed a definite, typical scar. In these four cases the greatest interval from the last successful vaccination to the contraction of small-pox was twenty-seven years; the shortest interval was twenty years, the intervals in the two remaining cases being twenty-six and twenty-four years, respectively. To find but four cases in a series of a thousand contracting small-pox after previous successful vaccination, and to find the period of immunity ranging from twenty to twenty-seven years, naturally leads to the conclusion that the protection afforded by successful vaccination lasts longer than shown by the records of Dr. Lescohier. This conclusion might be changed by observation on a much larger series of cases, and we might find a much shorter interval between successful vaccination and the contraction of small-pox. I wish to mention an interesting case in which small-pox developed a second time in a person who had previously had the disease. The patient, a boy, aged 9, when six months old, contracted a severe attack of small-pox from his mother. The mother gave a clear and definite history of this illness. Eight and a half years later the boy was exposed to small-pox a second time and contracted the disease. I saw him during convalescence from the second attack, with the unmistakable signs of small-pox—the dying lesions on his body and the hard, brown scabs beneath the skin of his palms. The second attack was mild, and there were but ten or fifteen lesions on the entire body. I mention this case to add it to the few previously recorded where a person has developed small-pox a second time. The second attacks of small-pox, as a rule, develop many years after the initial attack. This case seems unique, in that the patient developed the second attack so comparatively soon after the first illness.

Dr. V. C. Vaughan, Jr., Detroit: At one time we were unfortunate enough to have a mild epidemic of small-pox in the engineering department of the University of Michigan; in fact, it was so mild that it was not ascertained until we found one of the students picking off some scales, and on subsequent examination found that he had been afflicted with small-pox. We went through that department and found that every unvaccinated person had had small-pox in this very mild form. Another thing about which I wish to speak is the work which has been done in connection with revaccinations and the character of the reaction which we obtained in revaccination. I understand—although Dr. Lescohier's experience is much greater than mine with reference to this matter—that it is seldom that we get the typical reaction which we obtain in the original primary vaccination. Von Pirquet has done a great deal of work on accelerated vaccinations and he has shown that revaccination may be accomplished—may actually represent a true vaccination—and still not be noticed by the attending physician, who is looking for the development of the typical lesion. According to the theory of sensitization advanced by Dr. Vaughan, the vaccine body causes a reaction in the body of the person that leads to the production of a ferment, which subsequently causes cleavage of the vaccine body. This same ferment, of course, causes cleavage of the small-pox organism when it gains entrance to the body, and in that manner renders the patient immune. If such a person is revaccinated, if there is a large amount of ferment present the vaccine virus is attacked immediately, before it has had time to grow and multiply, and we have no reaction. If a certain amount of ferment is present, but not sufficient to attack it at once, then we have a mild, but an accelerated, reaction. I think physicians usually vaccinate themselves every time they see a case of small-pox. I certainly do, and I have had a take only once, but on several occasions I have had a notable reddening and itching there. I have said—and other physicians have made the same statement—that I would think it were a take, unless it had come on too early and subsided too rapidly. It really is one of these accelerated von Pirquet reactions, which may be used as a judge of the amount of immunity to the vaccine bodies.

Dr. C. A. Harper, Madison, Wis.: As a field man, I think my observations have strongly confirmed the results observed by Dr. Hensel and Dr. Vaughan. I have made personal observations of about eight hundred cases of small-pox, and only about two patients really had what we would call typical vaccination scars. All the others were either unvaccinated, or a few had scars which would be similar to a scar of infection, and not a typical vaccination scar. I believe that the appearance of small-pox in the field, after one successful vaccination—with the character of small-pox, particularly, that we have been having in this country—is an extremely rare occurrence. I am of the opinion that a revaccination will work much more frequently than attacks of small-pox in an individual who has been successfully vaccinated. I was vaccinated when a child, then I went into a line of work where I was to be frequently exposed to small-pox. I was vaccinated every year for a period of eight years. Each one of those years I was exposed to small-pox from fifty to two hundred times. In the eighth year the second vaccination took, but I have not had small-pox. I feel that the degree of immunity, with the small-pox that we find to-day—mild in character as it is—is almost absolute, after we have what we call a typical vaccination scar implanted on the body of the individual.

Dr. P. M. Hall, Minneapolis: I wish to confirm what Dr. Harper has said. Having been in field work during the past twelve years, and having seen probably about five thousand cases of small-pox, it has been my general observation that we do not see small-pox in those who show a typical vaccinal scar. I will go further than that, I never saw a death from small-pox—and have seen some cases of very virulent type—in a person who gave a history of ever having been vaccinated. With reference to the question of immunity, one peculiar case arose which I have never seen duplicated, showing the beneficial effects of vaccination after a definite exposure. The father of a family was removed on Sunday with definite small-pox, almost in the pustular stage, leaving behind a wife—pregnant and soon to be delivered—and two children, the wife and children never having been vaccinated. They were vaccinated immediately; the vaccinations took, the husband and father being removed the same day. Exactly two weeks from that date (the father still being away) the woman was confined, having a successful, working vaccination on her arm. Her baby took small-pox four days afterward and died.

Dr. C. Hampson Jones, Baltimore: My practical experience, and what I have read on the subject, has caused me to advocate that every one should be revaccinated at least once in ten years. I have had to deal with several outbreaks of small-pox—in 1899, 1901, 1905-6, and in 1912-13—not one of them with many cases, but all requiring a great deal of activity on our part to lessen the number of cases. With the exception of one man in 1899, in none of these outbreaks were there cases of small-pox in those who had been previously vaccinated, so far as the evidence of the vaccination scar showed. In the last outbreak, which began on Nov. 11, 1912, and the last case was on March 1, 1913, there were fifty-seven cases of small-pox, and not one of those patients had ever been previously vaccinated—there certainly was no scar. On the other hand, every one who had been successfully vaccinated and whom we knew to have been exposed to small-pox escaped. I am inclined to believe that we need careful and lengthy investigation to give us some idea as to the length of time that vaccination gives us protection. According to the number of removes from the original inoculation of the small-pox into the cow, the experimental evidence will show how each one will vary in the length of time of protection given to the unvaccinated individual. I also think that there undoubtedly is a great deal in the length of time that an unvaccinated and a vaccinated person are exposed to small-pox. For example: We segregate those—particularly among the negroes—who have been exposed to small-pox and were not previously vaccinated. It happens, over and over again, that those who have been exposed to small-pox for the first twenty-four or forty-eight hours of the eruption do not contract the disease. On the other hand, if there has been three, four or five days' exposure, it is quite certain that those who have been exposed will be stricken with small-pox.

Dr. Harold B. Wood, Rochester, Minn.: It might be well to correct an impression that seems to be circulated throughout the United States, that is, that Minnesota had stopped quarantining for small-pox a few years ago. We have had a number of letters asking how small-pox is controlled in this State. People have an erroneous impression that nothing whatever is done to segregate the people having small-pox. The people are not quarantined as the word is popularly understood in the East and South, but the people who have small-pox and who have not been vaccinated previous to one week before the exposure are actually quarantined within the household and the house is placarded. Compulsory vaccination is not legal here, but those who are exposed are given the option of vaccination within three days after exposure or of being quarantined three weeks. Where this quarantine with placarding has been carried out, there have been no return cases, so far as my records show. There has also been a marked decrease of small-pox in the State since the inauguration of the system. The disease, which has been epidemic throughout the country, has been carried on by cases which have not been discovered and are, in nearly all instances, extremely light cases. Some physicians and the majority of people do not realize that some of the extremely mild infections, where the vesicles are about one-tenth the diameter of the ordinary small-pox pustule, may be highly infectious. We have evidence that severe cases have been transmitted from extremely mild ones in which there are no pustules or scar formation.

Dr. Guy L. Kiefer, Detroit: There is a practical suggestion in the paper of Dr. Lescohier, urging us—by us I mean practical health officials —to require vaccinations in all cases of exposure more frequently than we have done in the past. My experience corroborates that of the field men, as they call themselves, who have spoken, and shows that immunity against small-pox, following vaccination, lasts apparently much longer than the immunity against a reinoculation with vaccine virus, and yet there are patients who are taken sick in less time than some of us suppose. For example, the first series of cases that I compiled was about eleven years ago, when we had an outbreak of mild small-pox, consisting of 1,023 cases, and of these, 862 patients had never been vaccinated successfully; 129 had been vaccinated, but in each case the period had been longer than five years, and in most of them longer than ten years; only 32 had been successfully vaccinated within a period of five years or a shorter time, according to their own testimony. We find, from the experiments given in Dr. Lescohier's paper, and from those he quoted, that about 50 per cent. of the persons will be susceptible to a second vaccination. It seems to me a practical suggestion that we might, in all cases of exposure, require the vaccination of all people who have not been successfully vaccinated within five years, and then, according to experiments, in at least half of them the vaccination will "take" and it will satisfy the community—it certainly will not do any harm—and it will be the limit of protection.

Dr. A. W. Lescohier, Detroit: I sincerely hope my paper did not carry the impression that I believed that persons who are successfully revaccinated after one or two years would be susceptible to small-pox. I think the evidence, not only in this country, but all over the world, shows that cases of small-pox in which there is a history of successful vaccination, even if ten or twelve years have elapsed, are rare. This is shown in Germany and Japan, where systematic revaccination is carried out in the eleventh year. The object in presenting these statistics was to indicate the advisability of a systematic revaccination, rather than to throw any discredit on vaccination as at present carried out—that is, infantile vaccination not followed up by revaccination. We cannot but believe that vaccination, even if only carried out in infancy, is the most effective means which we have in stamping out small-pox, but if it can be followed up after a reasonable number of years by reinoculation, the chances of completely controlling the disease will be much better.

ON CRYSTALLINE KOMBE-STROPHANTHIN.

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Commercial Strophanthus Kombe seed has frequently been found to contain seeds of different Strophanthus species. Mr. E. M. Holmes, F. L. S., the curator of the museum of the Pharmaceutical Society of London, observes:[1] "The Strophanthus leaves on the table were presented to the society's Herbarium some months ago by Mr. Lindsay, the curator of the Edinburgh Botanical Garden. They had been grown from some of the seed, used by Professor Fraser, apparently the greenish-brown kind. These leaves differed considerably from those specimens of Strophanthus Kombe in the Kew Herbarium, so that it appeared they were derived from a hairy leaved species, resembling Strophanthus hispidus, but not identical with it."

Twenty-two Kg. Strophanthus Kombe fruit (seed in pods) in very good condition were identified by Mr. Holmes, of London, as true Strophanthus Kombe Oliv. seeds. This statement is of great value, as there are more than twenty-nine species of Strophanthus[2]. The chemistry of Strophanthus seed Kombe, and in particular that of strophanthin, is in an uncertain state, due possibly to the fact that the strophanthin under examination was prepared from seeds other than the Kombe species, but generally to other causes, as will be shown. Commercial strophanthins vary considerably in their chemical reaction as well as their physiological activity.

To clear up these uncertainties and to determine whether a strophanthin of constant chemical, physical and physiological properties can be prepared, is the object of the experiments described in this paper. It includes also a summary of the most interesting work done on strophanthin and the methods for its preparation used by the different investigators with an account of the origin and characteristics of the seed used. A complete list of the chemical literature on Kombe-strophanthin (including gratus-strophanthin or ouabain[3] and hispidus strophanthin) is given at the end of the communication.

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[1] The Pharmaceutical Journal and Trans., 1887, 17, p. 754 and p. 755, also 1889, 20, p. 335.

[2] Holmes, The Pharmac. Journal and Trans., 1893, 23, p. 868 and p. 927. Hartwich, Archiv. der Pharm., 1892, 230, p. 401.

Fraser[4] was the first investigator to obtain an active principle which he called strophanthin from Strophanthus seed.

Some of the seeds, which Fraser used, were of a species resembling Strophanthus hispidus, but not identical with it.

Fraser's method for obtaining strophanthin was changed several times. The first process on which he based the second one is not accurately reported, as pointed out by Gerrard[5]. The first process seems to have been an extraction with alcohol, and a precipitation of the active principle with ether. The second process is as follows: The ether precipitate obtained in the earlier process is dissolved in water, tannic acid is added and then digested with recently precipitated oxide of lead. The digested material is extracted with alcohol and the solution precipitated by ether. The precipitate is finally dissolved in weak alcohol and through this solution carbon dioxide is passed for *several hours*, by which means the lead is completely removed. After filtration, the solution is evaporated at a low temperature and dried in vacuo over sulphuric acid.

The third process is the following: The powdered seeds were extracted with sulfuric ether, afterwards with 20 times their weight of rectified spirit. The spirit was distilled off. The active principle was precipitated by a solution of tannin from a

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[3] The following is a quotation from a publication of Hatcher and Bailey: "The clinical use of Strophanthus" in the Journal of the American Medical Association (Vol. 55, 1910, p. 1698): "Even such closely related bodies as strophanthin and crystalline ouabain, strophanthin, being methyl-ouabain, show different ratios of activity by subcutaneous and intravenous injections into the cat—." Because Strophanthin of the Pharmacopoeia of the U. S. is Kombe strophanthin, we are sure that the strophanthin Hatcher and Bailey refer to, must be Kombe-strophanthin. But then their statement cannot be true. As far as our knowledge goes about ouabain (or gratus strophanthin) this body has the formula $C_{30}H_{46}O_{12}+9H_2O$, and it is hydrolized according to the equation:

$$C_{30}H_{46}O_{12} + H_2O = C_6H_{12}O_5 + C_{24}H_{36}O_8.$$

$C_6H_{12}O_5$ being rhamnose anhydride (Arnaud, Comptes rendus 126 (1898), p. 1208). By the work of Feist, which is confirmed in this work, we know that Kombe-strophanthin is hydrolized to strophanthidin of the formula $C_{27}H_{38}O_7$. Without saying more about the properties of the cleavage product of ouabain (or gratus-strophanthidin) it will be clear that the statement that "Kombe strophanthin is methyl-ouabain," which is to be found in literature (Arnaud, Comptes rendus 107, 1888, p. 179, also Kohn and Kulisch Monatshefte 19, 1898, S. 385), cannot be true.

[4] The Pharmaceutical Jour. and Trans., 1873, 3, p. 523, 1887, 18, p. 69, 1889, 20, p. 206 and p. 328.

[5] The Pharmac. Jour. and Trans., 1887, 17, p. 923.

strong solution of the extract in water; the well washed tannate was thoroughly mixed with recently precipitated, carefully washed and moist oxide of lead, which was added in a quantity calculated to be necessary for the conversion of the tannin into tannate of lead; the mixture was digested for several days at a low temperature, and after it had been dried, it was thoroughly exhausted with alcohol. The alcoholic solution was generally evaporated to a syrupy consistence and repeatedly treated with oxide of lead until every trace of tannin had been removed. The product was now dissolved in dilute alcohol and any sediment removed by filtration, and through the clear and usually almost colorless solution a gentle stream of well-washed carbonic acid was passed *for two or three days*[6] in order to remove traces of lead. The solution was then evaporatd to dryness, and the residue dissolved in alcohol and filtered. Ether was added to the filtrate to precipitate the active principle. The precipitate was dissolved in absolute alcohol, which usually left a further slight sediment, and the clear alcoholic solution was finally dried by being placed in a partial vacuum over sulphuric acid. Strophanthin thus obtained is a colorless opaque and brittle substance, which under the microscope is found to consist of minute irregular crystalline plates. Strophanthin is intensely bitter and in solution in water or alcohol *is acid in reaction.* It melts at a temperature of (343° F.) 173° C. It burns without leaving a residue and contains no nitrogen. Strophanthin is decomposed by dilute acids yielding crystalline strophanthidin, and a syrup (acc. to Fraser glucose), which reduces Fehling's solution. The results of the combustion and its reaction with H_2SO_4 will be given later in the table.

Arnaud. The communication of *Arnaud*[7] is short yet interesting for our purpose; (a translation of the chemical part is as follows): The Strophanthus Kombe seeds in very good condition were furnished by Thomas Christy, of London. The following is the method of preparation. The seeds pulverized in the mill are extracted, using a reflux condenser with boiling 70% alcohol. After boiling some hours, the extraction is completed in a percolator. The alcoholic fluids are distilled on the waterbath to remove most of the alcohol, the distillation being finished in vacuo, leaving a certain amount of fluid. The oil floating on the top of the cooled extract is separated and the remaining fluid filtered. A small amount of a mixture of subacetate of lead and finely-powdered litharge is added, heated on the waterbath and filtered after cooling. The lead in solution is eliminated by hydrogensulphide and the clear fluid is concentrated at 50° to a thin extract. In twenty-four hours strophanthin will crystallize out. The crystals are filtered off, keeping the temperature at 50°. If the fluid is not too concentrated, the syrup can be separated by filtering and by placing the crystals on unglazed porcelain. The crystals can be purified by recrystallizing several times from *boiling water.*

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[6] Compare the preceding process.
[7] Comptes rendus, 1888, 107, p. 179.

The yield of crystallized Kombe-strophanthin is 4.5 gm. from 1 Kg. of seed, but a part of the strophanthin is kept in solution in the syrup. It is perhaps possible to recover this strophanthin by making the tannate.

Properties. Strophanthin is a white substance, very bitter, crystallizes in brilliant crystals grouped around a center of micaceous appearance, resembling cadmium iodide especially when suspended in water. These crystals are very porous and hold the water back easily. Strophanthin[8] forms a hydrate, which melts below 100° and loses its water in vacuum. By trying to recrystallize it, we find that the substance has become uncrystallizable, but if the substance is completely dehydrated in vacuo, it can be heated to 110° without alteration.

Strophanthin gives no residue when burned in the air. It has no sharp melting point, but softens at 165°, losing its opaqueness and quickly becoming brown. It acts on polarized light in watery solution (concentration 2.3%); (*a*) D=+30°

Cold water dissolves strophanthin slightly, 1 part to 43 parts of water 18° C. It is readily soluble in alcohol. It separates out of alcohol as a resin. It is insoluble in sulphuric ether, carbon disulphide and petroleum ether. Tannic acid precipitates it out of water solution. Strophanthin does not contain nitrogen. (The results of the combustion will be given in the table.)

Leopold Kohn and Victor Kulisch[9]. The seeds used by these authors to prepare strophanthin were examined by Hartwich, who declared the seeds to be identical with, or at least closely resembling, the seeds used to prepare the strophanthin with which Feist made his experiments and which were said to be Kombe seed.

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[8] The following is interesting as will be shown afterwards.

[9] Berichte 31, p. 514, and Monatshefte f. chemie, 19, p. 385.

The strophanthin of Kohn and Kulisch was compared with the strophanthin of Merck, which was prepared from hispidus seed. The preparations were found to be identical.

The method of preparation of Kohn and Kulisch is the same as that of Arnaud. The seeds were freed from comose hair and pounded, extracted in a Soxhlet apparatus with petroleum ether to remove the fatty oil, afterwards dried and then extracted with 70% alcohol. The filtered alcoholic extracts were precipitated with basic lead acetate and lead hydroxide and filtered. The filtrate was treated with hydrogen sulphide and filtered again. From the filtrate concentrated in vacuo the strophanthin separates out. Recrystallization from water proved to be the best method for purifying the product.

Properties. Under the microscope the preparation shows crystalline structure. It is white, of neutral reaction and does not contain nitrogen. It does not reduce Fehling's solution in the cold or by heating and is optically inactive or slightly levorotatory. Strophanthin is very hygroscopic. The water is hard to remove from strophanthin, which melts at 100° C. It is not easy to determine the melting point of strophanthin; the mean of several estimations for the carefully dried strophanthin is 179° C. (The results of combustion, other estimations and adopted formula of strophanthin will be compared later on in a table.)

When strophanthin is boiled with diluted acids a new insoluble crystalline body *strophanthidin* is formed and the filtrate reduces Fehling's solution.

Melting point, etc. (see table). The method of preparation of strophanthidin is as follows: 10 gm. strophanthin were dissolved in 200 cc. water and heated with 20 cc. HCl of spec. grav. 1.12 in a large flask with reflux condenser. When the fluid starts to boil the impure strophanthidin separates in fine yellow flakes. After heating fifteen minutes the fluid is cooled and filtered. The strophanthidin is washed until the acid reaction disappears and is recrystallized several times from hot alcohol. The final product appears in the form of fine white silky needles. Strophanthidin cannot be dried at 100°, as it is decomposed at this temperature.

Kohn and Kulisch also prepared strophanthidin by the method which Hardy and Gallois[10] used to prepare their "Strophanthin." The seeds were extracted with hydrochloric acid containing alcohol (1 part HCl in 10 parts alcohol). The filtered extract was diluted with water and evaporated until strophanthidin separated out. The filtered, well-washed sediment was recrystallized three times out of alcohol and gave pure white silky crystals, which proved to be identical with strophanthidin, prepared from strophanthin.

Feist[11] did not prepare strophanthin himself. The strophanthin used by Feist for his investigations was prepared by Boehringer and Sons (Waldhof) from Kombe Strophanthus seed by Fraser's process,[12] also a preparation of Schuchardt out of hispidus seed was found to be identical with the former preparation.

Properties. Strophanthin forms a fine white powder with a crystalline structure, or a brittle mass when obtained by evaporating a watery solution. It does not contain nitrogen and does not reduce Fehling's solution in the cold or by heating. It contains water in varying amounts. One cannot say whether the water is that of absorption or of crystallization. Feist gives the result of only one estimation: dried at 110° 7.18% H_2O. For the results of combustion and other data, see table.

Strophanthin was hydrolized with five times its weight of half percent hydrochloric acid. The flask was placed in a cold waterbath, and slowly heated. At 30° it was all dissolved and at 70-75° strophanthidin separated in small needles. After having kept the fluid some time at 75-80° it was cooled and filtered. The strophanthidin washed with a little sulphuric ether forms a white crystalline powder. The filtrate from the strophanthidin heated again at 75-80° separates out only a very small amount of the same product. Boiling the fluid gives very poor results, because resins are formed. Strophanthidin contains two molecules of water of crystallization. By drying only one and one-half molecules can be driven off.

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[10] Journal de Pharm., 1877, 25, p. 177, nearly the same in Comptes rendus 84, p. 261.

[11] Berichte 31, p. 534, 33, p. 2063 and p. 2069 and p. 2091.

[12] See the preceding abstract concerning the examination of the seed by Prof. Hartwich.

The water-free strophanthidin was prepared by recrystallizing several times from methylalcohol. A crystalline substance was thus obtained, consisting of one molecule strophanthidin and one molecule of methylalcohol. By drying this substance at 100° water-free strophanthidin was obtained. Strophanthidin hydrate melts at 169-170°, foaming at 176°, solidifying by cooling and melting again at 232°. The hydrate was examined optically and the crystals measured by Riva. The crystals are found to be monoclinic hemimorph. (See table.)

By studying the action of alkalies on strophanthidin Feist determined that strophanthidin is a dilacton $OHC_{25}H_{37}O_2\begin{matrix}/CO-O\backslash \\ \hline \backslash CO-O/\end{matrix}$. By the action of alkali on strophanthidin a salt of a dibasic acid is formed. By acidulating the solution of this salt and boiling, the original dilacton is not formed again, but *another lacton,* therefore, at least one OH is also present.

The second cleavage of strophanthin, *strophantobiose methylether,* was separated from the filtrate of strophanthidin. The combustion and the molecular weight estimation lead to the formula $C_{13}H_{24}O_{10}$. Strophantobiose-methylether was hydrolized by 1% sulphuric acid. In the fluid, resulting from this reaction, mannose was identified by the hydrazon and the osazon; rhamnose by the p-nitrophenyl rhamnosazon and by distilling the strophantobiosid with 30% sulphuric acid under which circumstances methylfurfurol is formed. Methylalcohol was identified by making a methoxyl estimation in strophantobiose-methylether. We are surprised that with the skill of Feist, the rhamnose was not obtained as a crystalline substance, as rhamnose crystallizes readily.

Heffter and Sachs[13]. Our investigations on the active principles of Kombe Strophanthus seed were already practically finished when the work of Heffter and Sachs appeared. Fortunately the publication of this paper was postponed so that we are able to incorporate the data of Heffter and Sachs. Their work is of especial importance to us as they studied the active principles of identified hispidus seed and unidentified Kombe seed, whereas we studied the active principles of identified Kombe seed and unidentified hispidus seed. The concordant results indicate that hispidus and Kombe seed which are on the market are pure, or that if they contain other species, these species do not contain other active principles.

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[13] Biochemische Zeitschrift 40 (1912), p. 83.

Heffter and Sachs used for the preparation of strophanthin the method of Thoms (Berichte 31, p. 271). The fat-free, powdered, identified Strophanthus hispidus seeds were exhausted with hot 94% alcohol. The alcohol was distilled off and the residue taken up with hot water. To the slightly acid colored turbid solution lead acetate in excess was added. Hydrogensulphide was passed through the filtrate in order to remove the lead; calcium carbonate in excess was added to the clear and nearly colorless acid solution and evaporated on the waterbath to a thin syrup and filtered. The strophanthin was at last salted out by a large amount of ammonium sulphate, and the strophanthin obtained was further purified by repeated extraction with 94% alcohol, and at last by precipitating from the absolute alcoholic solution with ether. In this way a yield of about 1.7% pure amorphous hispidus strophanthin was obtained. Kombe Strophanthus seed obtained in the open market gave 0.4-0.5% of crystalline Kombe strophanthin (which separated by evaporating the solution with calcium carbonate) and gave further an amorphous strophanthin, which was found to be apparently identical with amorphous hispidus strophanthin.

The figures of the analyses of these strophanthins obtained by Heffter and Sachs are compared in the table.

It is worth noting that Heffter and Sachs, using the modified method of Fromme (Hager's Handbuch der pharmac. Praxis Ergaenz band S. 669, 1908), for the quantitative strophanthidin determination, obtained values higher than ours, and probably nearer the theoretical.

OUR OWN RESEARCH.

Twenty-two Kg. Strophanthus Kombe seed in pods[14] gave 8.2 Kgs. seed (37.3%), 8.1 Kg. pods (36.8%), and 4.7 Kg. comose hair[15]. Helbing[16] who separated five pods gives the average; seeds, 37.36% pods, 37.61%—hairs, 25.02%. The 8.1 Kgs. seed were ground to a coarse powder[17] and extracted in a percolator with petroleum ether, to remove the fat. The yield was 5.6 Kg. fat-free powder and 2.5 Kg. green fatty oil (30.9%). Fraser[18] found 31.8% petroleum ether extract; 1.5 Kg. seed were twice percolated with 12 liters of 70% alcohol each time.[19] The alcohol of the first percolation was distilled off in vacuo. The thin extract, after standing a few days, deposited *crystals* of *strophanthin* which were suctioned off on a hardened filter. These crystals were recrystallized several times by dissolving in as little boiling water as possible and evaporating the solution at about 40°. By constant stirring at this temperature fine crystals were obtained, but the yield of crystalline strophanthin becomes smaller by every recrystallization, *e.g.*, 5 gm. pure crystalline strophanthin was recrystallized in this way and gave only 2 gm. crystalline strophanthin and 3 gm. of a white amorphous substance. The amorphous substance is more readily soluble in water than the crystalline strophanthin.

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[14] The seed, as has been previously mentioned, was found by Prof. E. M. Holmes to be seed of Strophanthus Kombe Oliv

While the crystalline strophanthin is *neutral*, the amorphous substance is in an aqueous solution *acid* in reaction. As will be shown later the estimation of the water of crystallization and the combustion of the crystalline strophanthin give figures which agree with the calculated figures for the formula $C_{40}H_{56}O_{15}+3H_2O$, while the estimations of the amorphous substance lead to the assumption that the amorphous substance is either a monobasic acid of the formula $C_{40}H_{56}O_{16}+3H_2O$ resulting from the action of water on one lacton group $C_{39}H_{56}O_{13}\underline{\diagup CO{-}O}+H_2O=C_{39}H_{56}O_{13}\genfrac{}{}{0pt}{}{\diagup COOH}{\diagdown OH}$ or a mixture of *e.g.* a monobasic acid, a dibasic acid and the original crystalline substance. It will be shown that the strophanthidin, which results by the splitting of strophanthin with acids, is identical with Feist's strophanthidin, which he showed to be a dilacton. A study of the salts which amorphous acid strophanthin can form will doubtless have a bearing on the constitution of the amorphous acid strophanthin. The figures obtained by the combustion and titration of amorphous acid strophanthin will be compared with the calculated figures for the formula of monobasic acid strophanthin of the above given formula.

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[15] The comose hair was not gathered with the same care as seeds and pods, because the hairs contained no strophanthin, and the separating of the seeds being rather disagreeable work, the dust affecting the mucous membrane of the nose, the separating was done in a well-ventilated room.

[16] The Pharmaceutical Journal and Trans., 1887, 17, p. 748.

[17] It is easier, as we afterwards found, to pound the seeds and after one extraction with petroleum ether to grind the extracted mass to a fine powder and extract it again to remove all the fatty oil, for it is impossible to grind to a fine powder the seeds containing fats.

[18] The Pharmaceutical Journal and Trans., 1889, 20, p. 329.

[19] Physiological testing, by Mr. O. E. Closson, showed that four successive extractions, made in the above-mentioned proportion, removed in the first percolation 86.5%, in the second percolation 9.4%, in the third percolation 2.4%, and in the fourth percolation 1.7% (considering 100% to be the whole amount of active substance extracted by these four percolations). Therefore, nearly all active substance is extracted in the first and second percolation.

The reversion of the amorphous acid into the crystalline lacton could not be accomplished. It is interesting to note that the *figures obtained by the combustion of the amorphous body are the same as those obtained by Arnaud and by Kohn and Kulisch for their strophanthins.*

The following method of preparation, which is similar to the method of Arnaud (the recrystallization of the impure strophanthin being the only differences), was afterwards adopted, because the alcoholic extract did not always crystallize and Arnaud's method gives a better yield.

Crystalline strophanthin as we have shown can be obtained without any chemical purification from the alcoholic extract. It is thus possible to determine whether a certain chemical method of preparation of strophanthin from Strophanthus Kombe seed, gives a yield of naturally occurring crystalline strophanthin or of a derivative of strophanthin.

Method of preparation. 1.5 Kg. ground, fat-free Strophanthus seed was percolated with 12 liters 70% alcohol and the percolate was distilled off in vacuo until about 1 liter fluid remained. To this fluid sufficient lead subacetate solution (Liquor plumbi subacetatis, U. S. P.) was added, to obtain an easily filtering mixture. The filtrate is a clear yellow fluid. The excess of lead was removed by hydrogen sulphide and the clear filtrate was evaporated at 40°-45° with constant stirring. Until the fluid becomes concentrated it is important that it be kept alcoholic by frequent addition of a little alcohol. When the fluid has become a thin extract, the alcohol must be evaporated as much as possible. It will then crystallize readily. The crystals are separated on a hardened filter of large surface by suction. The recrystallization is made in the following manner to avoid conversion into the amorphous body: The crystals are dissolved by placing them in a dish with a small amount of 94% alcohol and heating to 40-45° and stirring occasionally. After having filtered the solution, the alcohol is now evaporated to a thick extract at this temperature and water is added until a thin extract is obtained. With constant slow stirring with a motor, the extract is again evaporated to remove the rest of the alcohol. The extract will then crystallize readily. As was observed by Arnaud, the alcohol cannot be used alone to recrystallize, because it leaves strophanthin as a varnish. This recrystallization is repeated until strophanthin is obtained with the properties, which will be described.

Another method of preparation was carried out as proposed by Bourquelot and Herissey[20] to exclude enzyme action, by adding 2.6 Kg. fat-free ground seed to 15 liters boiling alcohol of 94%. After boiling for twenty minutes the hot fluid was filtered off and the alcohol evaporated in vacuo; this gave an extract from which crystalline strophanthin identical with the crystalline strophanthin made by the previously described method was obtained.

Crystalline strophanthin, identical with the former described crystalline strophanthin, was also obtained by the method, described, from Strophanthus Kombe seed, regularly purchased for the manufacture of Tr. of Strophanthus. The first crystalline strophanthin will be called: strophanthin (ident.), the second: strophanthin (trade). No difference was found between them.

Properties of crystalline strophanthin. Kombe strophanthin (ident. and trade) is a white crystalline substance, showing under the microscope fine needles or long plates.

It contains three molecules of water of crystallization, which are readily given off in a vacuum desiccator more quickly in vacuo at 80°. By placing the dehydrated crystals in a chamber containing moist air the water is taken up again. In a vacuum at 100° C. or 105-110° C. no more water is given off than in vacuo at 80° C. Found by drying at 80° C. and 105-110° C. in vacuo for Strophanthin (ident.): 6.0%-6.5%-6.3%. In strophanthin (trade) 7.1 and 7.0%. Calculated for $C_{40}H_{56}O_{15}+3H_2O$:6.5% H_2O.

Melting point. The substance melts in its water of crystallization at 158-165° C., becoming a turbid mass. Dried, it melts at 178-179° C. Strophanthin (trade) gave the same results, and by mixing them together the same results were obtained.

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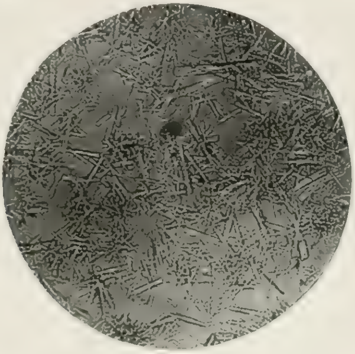
[20] Comptes rendus, 133, p. 690.



Crystalline Kombé-strophanthin.

Specific rotation was determined in 94% alcoholic solution of air-dried substance.

I. 1.000 gm. cryst. strophanthin (ident.) dissolved in 25 cc. 94% alcohol.

$$(a)_D = \frac{100a}{l\,c} = +31.6 \qquad l = 2 \qquad a = +2.53^\circ$$

II. 0.7500 gm. cryst. strophanthin (ident.) dissolved in 25 cc. 94% alcohol.

$$(a)_D = \frac{100a}{l\,c} = +31.6 \qquad l = 2 \qquad a = +1.90^\circ$$

III. A determination was also made in absolute (99.5%) alcohol.
0.7500 gm. cryst. strophanthin (ident.) dissolved in 25 cc. 99.5% alcohol.[21]

$$(a)_D = \frac{100a}{l\,c} = +31.3 \qquad l = 2 \qquad a = +1.88^\circ$$

IV. A determination was also made in watery solution (to compare with Arnaud's value).

a. 0.200 gm. cryst. strophanthin (ident.) dissolved in 25 cc. water.

$$(a)_D = \frac{100a}{l\,c} = +28.7 \qquad l = 2 \qquad a = +0.23^\circ$$

b. 0.7500 gm. cryst. strophanthin (trade) dissolved in 25 cc. 94% alcohol.

$$(a)_D = \frac{100a}{l\,c} = +30.5 \qquad l = 2 \qquad a = +1.83^\circ$$

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[21] For differences in rotation of alkaloids in 94% alcohol and absolute alcohol. See Jour. of Chem. Society (London), 1910, 97, p. 1328.

Combustion.

I. 0.2670 gm. cryst. strophanthin (ident.) dried at 80° in vacuo gave 0.1947 gm. H_2O and 0.6073 gm. CO_2.

II. 0.2318 gm. cryst. strophanthin (ident.) dried at 105-110° in vacuo gave 0.1667 gm. H_2O and 0.5291 gm. CO_2.

III. 0.2675 gm. cryst. strophanthin (ident.) dried at 105-110° in vacuo gave 0.1846 gm. H_2O and 0.6047 gm. CO_2.

IV. 0.2520 gm. cryst. strophanthin (trade) dried at 105-110° in vacuo gave 0.1802 gm. H_2O and 0.5680 gm. CO_2.

V. 0.2597 gm. cryst. strophanthin (trade) dried at 105.110° in vacuo gave 0.1872 gm. H_2O and 0.5863 gm. CO_2, or in percent:

	S. ident.			S. trade.		Aver. S. ident.	Aver. S. trade.	Calculat. f. $C_{40}H_{56}O_{15}$
	I.	II.	III.	IV.	V.			
C.....	62.03	62.25	61.65	61.47	61.57	61.97	61.52	61.82
H....	8.17	8.06	7.73	8.01	8.08	7.98	8.04	7.27

An aqueous solution of cryst. strophanthin has the following properties: Heated with a small amount of a mineral acid and made alkaline afterwards, it readily reduces Fehling's solution.

Crystalline strophanthin can be boiled with glacial acetic acid without producing a substance capable of reducing Fehling's solution. A trace of crystalline strophanthin, when added to sulphuric acid first turns dark green and then brownish.[22]

It gives a white precipitate with tannic acid solution and no precipitate with lead acetate or basic lead acetate solution.

Properties of amorphous acid strophanthin. Acid strophanthin was not obtained in a recognizably crystalline form. It is difficult to determine how much water is present as a hydrate, since amorphous acid strophanthin is hygroscopic. This water is readily given off in vacuo at 100° C., no more water being given off in vacuo at 105-110° C. Found by drying in vacuo at 105-110° 5.9%, in other samples 7.7% and 8.0% of water. Calculated for $C_{40}H_{58}O_{16}+3H_2O$ 6.37%.

Melting point. Acid strophanthin softens at about 100° C. in its hydrate water and melts at 165-170° C. The dried substance softens at 160° C. and melts about 180° C.

Specific rotation was determined in watery solution of air-dry substance 0.400 gm. dissolved in 25 cc. water.

$$(a)\qquad D=\frac{100a}{l\,c}=+20.6 \qquad\qquad l=2 \qquad a=+0.33^\circ$$

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[22] Reaction of Helbing. The Pharmac. Jour., 1877, 17, p. 924.

Combustion.

I. 0.3375 gm. amorph. acid strophanthin (ident.) dried at 105-110° in vacuo gave 0.2333 gm. H_2O and 0.7477 gm. CO_2.

II. 0.2553 gm. amorph. acid strophanthin (ident.) dried at 105-110° in vacuo gave 0.1717 gm. H_2O and 0.5672 gm. CO_2.

III. 0.2316 gm. amorph. acid strophanthin (trade) dried at 105-110° in vacuo gave 0.1609 gm. H_2O and 0.5145 gm. CO_2.

IV. 0.2645 gm. amorph. acid strophanthin (trade) dried at 105-110° in vacuo gave 0.1873 gm. H_2O and 0.5899 gm. CO_2, or in percent:

	Ident.		Trade.		Aver. trade.	Aver. ident.	Calculat. f. $C_{40}H_{58}O_{16}$
	I.	II.	III.	IV.			
C..........	60.42	60.59	60.58	60.82	60.70	60.50	60.42
H..........	7.75	7.50	7.79	7.94	7.86	7.62	7.36

Molecular Weight of Crystalline Kombe Strophanthin and Other Kombe Strophanthins.

Feist (Berichte 31 (1898), p. 536 and 33 p. 2075), determined the molecular weight of Kombe strophanthin. In his first preliminary report Feist said: "The chosen formula $(C_2H_3O)_{16}$ is also supported by the cryoscopic molecular weight determination in water. Calculated 688, found in the average 678 and 680. Notable molecular association occurs with increasing concentration."

The figures found by the determination are reported in his following paper in which Feist says: "The cryoscopic molecular weight determinations can only be determined in water (c=18.9) and give very uncertain results, calculated $C_{40}H_{66}O_{19}$=850.

I. Used air-dry strophanthin hydrate in tablets.

Substance.	Depression.	Mol. Weight Found.	Average Mol. Weight.
0.0956	0.030	572.8	678.5
0.2520	0.069	656.0	
0.3997	0.089	806.8	

The amount of water used was 10.52 gm.

II. Used strophanthin, which was dried several months over sulphuric acid, hygroscopic powder.

Substance.	Depression.	Mol. Weight Found.	Average Mol. Weight.
0.3155	0.079	614.1	680.8
0.6508	0.150	655.3	
1.2178	0.249	752.1	

The amount of water used was 12.29 gm.

The following are the figures obtained by our own research.

The strophanthins used for the experiments were:

I. Crystalline Kombe strophanthin; II, acid amorphous Kombe strophanthin (obtained by the action of water on crystalline Kombe strophanthin); III, amorphous strophanthin Merck.

Ia. Crystalline Kombe strophanthin.

Substance.	Depression.	Mol. Weight Found.	Calculated for $C_{40}H_{56}O_{15}$
0.0874	0.023	481	776
0.1785	0.037	611	

The amount of water used was 15.01 gm.

Another determination was made with crystalline Kombe strophanthin.

Ib. Crystalline Kombe strophanthin.

Substance.	Depression.	Mol. Weight Found.	Calculated for $C_{40}H_{56}O_{15}$
0.0517	0.012	551	776
0.1451	0.032	577	
0 2344	0.053	563	

The amount of water used was 14.91 gm.

II. Acid amorphous strophanthin.

Substance.	Depression.	Mol. Weight Found.	Calculated for $C_{40}H_{58}O_{16}$
0.1865	0.061	390	794
0.3940	0.100	503	
0.5223	0.139	486	

The amount of water used was 14.9 gm.

III. Amorphous Kombe strophanthin-Merck.

Substance.	Depression.	Mol. Weight Found.
0.1407	0.030	596
0.2740	0.054	645
0.4246	0.081	666

The amount of water used was 14.95 gm.

These figures are in agreement with those of Feist for the same concentration. The last determination of the first experiment of Feist is undoubtedly an error. From these figures we observe that with higher concentration the molecular weight increases, therefore the figures of Feist's second experiment become close to the theoretical (776). Feist's explanation of this behavior was that the uncertain results are due to notable molecular *association* by increasing concentration. Through our recent investigation of the conversion of crystalline Kombe strophanthin into the acid amorphous strophanthin we are able to give a better explanation, viz.: *dissociation*. This is already indicated by the figures of Feist in which all his molecular weight determination fall well below the calculated.

The molecular weight was therefore determined in a non-ionizable fluid, which at the same time possesses sufficient solubility for strophanthin, in order to obtain the correct values. These fluids, alcohol, acetone, etc., could only be used conveniently by determining the increase in boiling point. The Beckman apparatus was used, in which heating is accomplished by circulating vapor (Zeitschr. f. physik. Chemie, 40, 1902, p. 133).

The determination was made with alcohol as a solvent. Beckman determined the constant (molecular increase for 100 cc.) =15.6°.

I*a*. Crystalline Kombe-strophanthin.

Cc. of alcohol.	Gm. Subst.	Observed increase.	Gm. Subst. in 100 cc.	Found Mol. W.
14.1	0.3020	0.042	2.1464	797
15.7	0.3020	0.028	1.2850	716

I*b*. Crystalline Kombe-strophanthin.

Cc. of alcohol.	Gm. Subst.	Observed increase.	Gm Subst. in 100 cc.	Found Mol. W.
12.7	0.3947	0.058	3.1080	836

The average molecular weight found for crystalline Kombe Strophanthin 1/3(797 + 716 + 836) = 783. Calculated for $C_{40}H_{56}O_{15}$ = 776.

II. Amorphous Kombe-strophanthin Merck.

Cc. of alcohol.	Gm. Subst.	Observed increase.	Gm. Subst. in 100 cc.	Found Mol. W.
11.6	0.3116	0.044	2.6862	952
12.7	0.3116	0.037	2.4535	1034
14.4	0.3116	0.032	2.1639	1055

The average molecular weight found for Kombe strophanthin Merck 1/3(952 + 1034 + 1055) = 1014. The barometer stood constant during the experiments.

We believe that crystalline Kombe strophanthin contains two lacton groups, which may be different from the strophanthidin grouping, in that there are OH radicals in the sugar which may interact with the formation of different lacton complexes and of internal ethers, thus accounting for the two less molecules of water in the molecule of strophanthin than would otherwise be expected. When dissolved in water both of these lacton groups hydrolyze to a certain extent into acids, which by their ionization give different molecular weight values, depending on the concentration. Therefore, by greater dilutions the apparent molecular weight calculated, according to the formula of Raoult, is smaller, whereas by increasing concentration the molecular weight approaches the molecular weight of the calculated formula. For the acid amorphous strophanthin we believe that the molecule has undergone a rearrangement by which one of the lacton groups is converted permanently into an acid, which remains an acid independent of the concentration.

A simple reaction demonstrated that amorphous Kombe strophanthin Merck must contain a certain amount of acid amorphous strophanthin. Three pieces of blue or neutral litmus paper are moistened with water. A small amount of crystalline Kombe strophanthin is put on one, on the second a small amount of acid amorphous Kombe strophanthin, and on the third piece of litmus paper a small amount of amorphous Kombe strophanthin Merck. The papers indicate (by looking on the other side) that only crystalline Kombe strophanthin is neutral, whereas the other samples behave as acids.

Contrary to Feist's explanation, we find that the lower figures obtained with the cryoscopic molecular weight determination are due to a dissociation of the molecule.

By using alcohol with the ebullioscopic method nearly theoretical figures were obtained for the molecular weight of crystalline Kombe-strophanthin.

Titration of amorphous acid strophanthin with N/10 sodium hydroxide, phenolphthalein as an indicator.

I. 0.5000 gm. amorph. acid strophanthin (air dry) was dissolved cold in 10 cc. N/10 sodium hydroxide and titrated back with N/10 oxalic acid. Found: that 3.2 cc. N/10 sodium hydroxide was necessary to neutralize the strophanthin.

II. The same investigation repeated with 0.500 gm. air-dry amorph. acid strophanthin gave as a result that 3.1 cc. N/10 sodium hydroxide was necessary to neutralize the strophanthin.

III. 0.5000 gm. amorphous acid strophanthin (air dry) was dissolved in 10 cc. N/10 sodium hydroxide and heated until the fluid boiled. The cooled solution was titrated back with N/10 oxalic acid. Found: that 5.5 cc. N/10 sodium hydroxide was necessary to neutralize the strophanthin. Repeating the boiling with more alkali gave only a slight increase of the neutralizing value.

IV. The same investigation as III repeated with 0.5000 gm. air-dry amorph. acid strophanthin showed that 5.4 cc. N/10 sodium hydroxide was necessary to neutralize the strophanthin.

Found in the cold for 0.5000 gm. acid strophanthin: 3.2 cc. and 3.1 cc. N/10 NaOH.

Found by heating for 0.5000 gm. acid strophanthin: 5.5 cc. and 5.4 cc. N/10 NaOH.

Calculated for 0.5000 gm. monobasic strophanthin ($3H_2O$) 5.88 cc. N/10 NaOH.

Amorphous acid strophanthin is much more soluble in water than crystalline strophanthin. A watery solution of amorphous acid strophanthin has the following properties:

It slowly reduces Fehling's solution by boiling (a fresh aqueous solution of crystalline strophanthin dissolved in the cold does not reduce Fehling's solution by boiling).

Heated with a small amount of a mineral acid and made alkaline afterwards; it readily reduces Fehling's solution. It gives a white precipitate with tannic acid solution and no precipitate with lead acetate or basic lead acetate solution.

A trace of amorphous acid strophanthin, when added to sulphuric acid, first turns greenish-brown and then brown.

When a 12% solution of hydrochloric acid containing crystalline or amorphous strophanthin is boiled no appreciable amount of furfurol or methylfurfurol is formed.[23]

0.2000 gm. pure rhamnose ($C_6H_{14}O_6$) was distilled with 500 cc. 12% hydrochloric acid from a round flask, which was connected with a condenser by a ground joint. The distilling fluid was gathered in an Erlenmeyer flask, which had a mark indicating 100 cc. A small piece of unglazed porcelain was added to the boiling fluid to prevent bumping. The round flask was heated in an air bath. After about 4 hours the distillation was ready and to the clear distillate a filtered solution of 0.300 gm. pure phloroglucin in about 15 cc. warm 12% HCl was added and the Erlenmeyer flask was kept dust-free for 15-18 hours. Then the methylfurfurol-phloroglucid, which had settled as a red precipitate, was filtered on a weighed quantitative filter, which had been dried at 97-100°. The precipitate was washed with 150 cc. water and the filter with precipitate dried for 3½-4 hours at 97-100° and weighed. 0.200 gm. rhamnose ($C_6H_{14}O_6$) gave 0.1310 gm. methylfurfurol-phloroglucid or according to the formula of Ellett and Tollens. (Journal f. Landwirtschaft 1905, p. 13.)

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[23] Concerning the furfurol and methylfurfurol estimations:
Kröber: Journ. f. Landwirtschaft, 1900, p. 379.
Ellett and Tollens: Ber. 38, p. 492, Journ. f. Landw., 1905, p. 13.
Mayer and Tollens: Journ. f. Landw., 1907, p. 268, Ber. 40, p. 2441.

Rhamnose$=$Ph.$\times$1.65$-$Ph$^2\times$1.84$+$0.010.

When Ph$=$amount of phloroglucid.

we find 0.209 gm. rhamnose.

The method was tried out on ouabain (gratus strophanthin), which according to Arnaud and also to Thoms has the formula $C_{30}H_{46}O_{12}+9\ H_2O$. Arnaud could separate crystalline rhamnose by splitting ouabain with acids. A quantitative estimation of rhamnose was made by Arnaud by titrating the fluids remaining by hydrolysis according to Soxhlet. Arnaud[24] found in this way 24.2% $C_6H_{14}O_6$ in $C_{30}H_{46}O_{12}+9\ H_2O$ calculated for 1 molecule $C_6H_{14}O_6$ 23.9%.

0.960 gm. air-dry ouabain was distilled in the above described manner and gave 0.1540 gm. methylfurfurolphloroglucid or according to the formula of Ellett and Tollens found: 0.2205 gm. rhamnose in 0.960 gm. $C_{30}H_{46}O_{12}+9\ H_2O$ or 22.9%.

The method was now applied to crystalline and acid amorphous strophanthin. 0.750 gm. air-dry crystalline strophanthin gave 0.0074 gm. brownish phloroglucid. Calculating this as methylfurfurolphloroglucid we get according to the formula of Ellett and Tollens 0.0211 gm. rhamnose ($C_6H_{14}O_6$) in 0.750 gm. air-dry crystalline strophanthin or 2.9%. 0.850 gm. air-dry amorphous acid strophanthin gave 0.0070 gm. brownish-red phloroglucid. When we figure that it is methylfurfurolphloroglucid, we get according to the formula of Ellett and Tollens

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[24] Comptes rendus 126 (1898), p. 1208.

0.0215 gm. rhamnose ($C_6H_{14}O_6$) in 0.850 gm. amorphous strophanthin, or 2.5%. If air-dry crystalline strophanthin ($C_{40}H_{56}O_{15}$ $+3\ H_2O$) should contain 1 molecule rhamnose the percentage $C_6H_{14}O_6$ would be 21.9%, and for the air-dry amorphous acid strophanthin ($C_{40}H_{58}O_{16}$ $+3\ H_2O$) : 21.4%.

We can conclude, therefore, that no rhamnose is present in Kombe strophanthin.

According to Feist rhamnose and d-mannose should be present in Kombe strophanthin. If 2 molecules of d-mannose are contained in crystalline strophanthin then 0.750 gm. $C_{40}H_{56}O_{15}$ $+3\ H_2O$ should contain 0.325 gm. mannose and 0.850 gm. $C_{40}H_{58}O_{16}$ $+3\ H_2O$ should contain 0.359 gm. mannose. Therefore, 0.340 gm. d-mannose was distilled with 12% HCl to allow a comparison with the results of the former estimations. When 0.300 gm. phloroglucin was added to the distillate of 0.340 gm. mannose with 12% HCl *no precipitate* was visible after 18 hours. Therefore, d-mannose cannot be the reason of the formation of the small yield of phloroglucid from strophanthin.

Strophanthidin. The strophanthidin prepared by Kohn and Kulisch from their strophanthin is different from the strophanthidin prepared by Feist. By splitting crystalline strophanthin by the method of Kohn and Kulisch or by other methods, or splitting amorphous acid *strophanthin with* 1% H_2SO_4, *the same strophanthidin was always obtained and this strophanthidin proved to be identical with that described by Feist.*

Strophanthidin was prepared from crystalline strophanthin by the method of Kohn and Kulisch. (See the description of this method above.)

It separated out in yellow crystals, which after recrystallizing three times from hot alcohol and concentrating the solution, separated in fine, large, colorless crystals, which showed under the microscope the same form as the picture of strophanthidin given by Feist. The following procedure gives a better yield than the method of Kohn and Kulisch: To 100 cc. boiling 1% HCl 5 gm. crystalline strophanthin in fine powder was added. After dissolving the strophanthin the fluid was cooled and strophanthidin separated quickly in fine white crystals, which were filtered by suction and washed with water. After two crystallizations from hot alcohol nice large crystals can be obtained. Amorphous acid strophanthin separates a dark resin when heated with 2% HCl. The best method of splitting amorphous acid strophanthin was found to be boiling with 1% H_2SO_4. 6 gm. amorphous acid strophanthin was boiled with a reflux for one-half hour with 50 cc. 1% H_2SO_4. The yellow separation was repeatedly crystallized from hot alcohol and gave the same strophanthidin as prepared from crystalline strophanthin, therefore, by the action of sulphuric acid, the acid group goes over to a lacton group.

Properties of strophanthidin. Under the magnifying glass strophanthidin shows the same shape of crystals as shown in the picture of strophanthidin given by Feist. It contains one molecule of water of crystallization. Feist had trouble in drying the substance, and it was only by obtaining crystals of a methyl alcohol containing strophanthidin, which gave off readily the methyl alcohol at 100° C., that he could establish the formula for dry strophanthidin. Feist, and also Heffter and Sachs, did not dry in a heated vacuum, but only at ordinary pressure. By drying in vacuo at 110-115°, we could readily obtain crystalline water-free strophanthidin. Also at lower temperature (105° in vacuo) the water is given off, but slowly. In moist air the water is taken up again. Found: 3.56%, 3.50% and 3.02% calculated for $C_{27}H_{38}O_7+H_2O$: 3.66% H_2O.

Melting point. Strophanthidin melts at about 120° C. in its water of crystallization to a turbid mass and melts at about 170° C. The dried substance melts as Feist describes at 169°-170° C., foaming at 180° C.; it becomes solid by cooling and then melts at 232° C.

Specific rotation. 1.008 gm. air-dry strophanthidin (from crystalline strophanthin) was dissolved in 25 cc. methyl alcohol.

$$(a)_D = \frac{100a}{l\,c} = +44.26 \qquad l = 2, \quad a = +3.57^\circ$$

0.3300 gm. air-dry strophanthidin (from amorph. acid strophanthin) was dissolved in 25 cc. methylalcohol.

$$(a)_D = \frac{100a}{l\,c} = +44.26 \qquad l = 2, \quad a = +1.17^\circ$$

Feist found for 0.5043 gm. strophanthidin dissolved in 25 cc. methyl alcohol

(*a*)

$D = -45.45$

Combustion.

I. 0.2196 gm. air-dry strophanthidin (from cryst. strophanthin) gave 0.1598 gm. H_2O and 0.5300 gm. CO_2.

A second combustion was made of air-dry strophanthidin (from cryst. strophanthin trade) which was not purified by recrystallization from alcohol, but was used as it is obtained in splitting the crystalline strophanthin according to Kohn and Kulisch.

After having been thoroughly washed with water it was dried in air.

II. 0.2198 gm. air-dry strophanthidin (not recrystallized) gave 0.1660 gm. H_2O and 0.5312 gm. CO_2.
or in percent:

	I	II	Calc. f. $C_{27}H_{38}O_7 + H_2O$
C	65.82	65.91	65.80
H	8.16	8.46	8.20

Thus it is apparent that recrystallization does not alter the constitution.

III. 0.2054 gm. strophanthidin (from cryst. strophanthin) dried at 110-115° in vacuo gave 0.1499 gm. H_2O and 0.5164 gm. CO_2.

IV. 0.2330 gm. strophanthidin (from amorph. acid strophanthin) dried at 110-115° in vacuo gave 0.1770 gm. H_2O and 0.5845 gm. CO_2; or in percent:

	III	IV	Calc. f. $C_{27}H_{38}O_7$
C	68.57	68.41	68.30
H	8.18	8.01	8.09

Quantitative estimation of strophanthidin in crystalline strophanthin.

0.5000 gm. air-dry crystalline Kombe strophanthin was placed in a 200 cc. Erlenmeyer flask with 25 cc. 1% H_2SO_4 and boiled one hour with a reflux condenser. The separated strophanthidin was caught on a quantitative filter. The filtrate and wash water (about 50 cc.) was distilled to 25 cc. and boiling continued with a reflux for one-half hour. The separated strophanthidin was caught on the same filter paper and this process once more repeated. The total yield of strophanthidin was dried in vacuo, giving 0.2638 gm., or 52.76%.

0.500 gm. of air-dry crystalline Kombe strophanthin was placed in a 200 cc. Erlenmeyer flask with 22 cc. of 2.2% HCl (twice the volume of the same strength HCl as used by Kohn and Kulisch) and boiled one-fourth hour with a reflux condenser, cooled and filtered; the filtrate concentrated to original volume and boiling continued with reflux condenser for one-fourth hour. After cooling, the separated strophanthidin was caught on the same filter and dried in vacuo, giving 0.2533 gm., or 50.46%.

Quantitative estimation of strophanthidin in amorphous Kombe strophanthin.

0.500 gm. air-dry amorphous Kombe strophanthin Merck was placed in an Erlenmeyer with 50 cc. 0.5% HCl and heated to 75° and further treated, according to the method of Feist, repeating the heating three times. Dried in a vacuo, 0.2218 gm. strophanthidin was obtained, or 44.36%.

0.5000 gm. air-dry amorphous Kombe strophanthin Merck was placed in an Erlenmeyer with 25 cc. of 1% H_2SO_4 and treated as the first determination on crystalline Kombe strophanthin. This gave 0.2234 gm. of vacuum dry strophanthidin, or 44.78%.

The largest yield of strophanthidin obtained by Heffter and Sachs from crystalline Kombe strophanthin (air dry) was 58%, from amorphous Kombe strophanthin 50%, and from hispidus strophanthin 45%.

Air-dry crystalline Kombe-strophanthin $C_{40}H_{56}O_{15}+3\ H_2O$ contains 57.11% anhydrous strophanthidin ($C_{27}H_{38}O_7$).

It is interesting to note that the yield of strophanthidin from amorphous Kombe strophanthin and from hispidus strophanthin is less than from crystalline Kombe-strophanthin, thus indicating (cf. the later pentose estimation in amorphous strophanthin) that the non-strophanthidin moiety is larger in hispidus and amorphous Kombe-strophanthin than in the crystalline Kombe strophanthin.

CRYSTALLINE KOMBE-STROPHANTHIN.

Table of Comparisons.

Strophanthin.	Fraser	Arnaud	Kohn & Kulisch. a)	Feist. b)	Heffter & Sachs.	Own investigations.	
						By spontan. crystal of alcoh. extr.	
How prepared.	Ppt. by tannic acid and dec. by PbO.	Removed impur. by basic lead.	Same as Arnaud.	Same as Fraser.	About same as Arnaud.	Cry. stroph.	Am. acid stroph.
Reaction with H_2SO_4	green	....	red	green	dark green	dark green	red-green
Melting point of dried str.	173°	165° not dried	179°	170°	177-181°	178-179°	180°
Average % of hydr. water.	....	....	....	7.48	....	6.3	6.9
Spec. Rot. of watery sol..	....	+30	inact. or neg.	+10.12	+28.72	+28.7	+20.6
How dried for comb.	....	110°	105-109°	100-105°	105-110°	105-110°	105-110°
Aver. % C....	55.43	60.54	60.57	56.17	61.93	61.97	60.50
Aver. % H....	7.56	8.00	7.71	7.36	7.64	7.98	7.62
Aver. % OCH_3	....	....	3.58	3.64	4.73	....	....
Mol. wgt......	....	....	....	679	....	783	....
Strophanthidin %	....	....	52.5	50-52	56-58	50-53	....
Strophanthidin prep. by action of	....	....	boil 2.4% HCl	0.5% HCl at 75°	0.5% HCl at 75°	boil 4% and 1% HCl	boil 1% H_2SO_4
Melt p. of dried	....	....	193°	169-170° foam 176°	169-173° foam 178°	170° foam 180°	170-180°
Aver. % hydr. water	....	....	....	7.0	....	3.6	3.3
Spec. Rot. alcoh. sol....	....	....	....	+45.45	+41.49	+44.26	+44.3
How dried f comb.....	....	....	in vac. + H_2SO_4	c)	vac. + H_2SO_4	vac. at 110°	vac. at 110°
Aver. % C....	....	....	71.07	68.32	66.44	68.57	68.41
Aver. % H....	....	....	8.63	8.02	8.07	8.18	8.01
Aver. % OCH_3	....	....	5.6	No OCH_3	No OCH_3	....	....
Mo wgt.......	....	....	....	474	....	....	....
Rhamnose estim.	....	....	....	1 mol. rham	....	none	none
OCH_3 in sugar	....	....	....	6.25	....	....	....

(a) Strophanthin prepared by Merck from hispidus gave Kohn and Kulisch the same results.
(b) Strophanthin prepared by Schuchardt from hispidus gave Feist the same result.
(c) Strophanthidin containing chrystalmethylalcohol was dried at 100°.

From this table, it seems very probable that Arnaud separated the same crystalline strophanthin as we did, but by recrystallizing it, converted it into the amorphous one, and using amorphous strophanthin for his combustion (supposing it to be a hydrate, which by drying could give off the water) came to wrong conclusions.

The strophanthin of Kohn and Kulisch has a different specific rotation and gives by splitting a different strophanthidin, which may be due to the method of cleavage.

It would be interesting to investigate other strophanthus species, or to prepare Kombe-strophanthin from Strophanthus Kombe seed at different stages of ripeness in an endeavor to obtain the strophanthin of Kohn and Kulisch. It may be stated here that the formula and cleavage equation which Feist[25] deduced from the data of Kohn and Kulisch best interpret their results.

The table shows the exact coincidence of our results with the analytical data of Heffter and Sachs; with the exception of the figures for the combustion of strophanthidin and the quantitative estimation of strophanthidin.

Heffter and Sachs seemed to have the same difficulty as Feist in obtaining an anhydrous strophanthidin. It is interesting to note that drying in a heated vacuum readily yields an anhydrous strophanthidin. This is shown by the agreement of the analytical data with the calculated figures and with the data obtained by Feist for his strophanthidin, which was obtained by driving off methyl alcohol from methyl alcohol containing strophanthidin.

The quantitative estimation of strophanthidin, applied by Heffter and Sachs, gives undoubtedly the best results.

When we compare the results of Feist with our own, the question arises: Are the differences due to the use of Fraser's method? By applying the method of Fraser on Strophanthus Kombe seed a difficulty arises, how to remove the lead and the nitrogen. The same trouble was mentioned by Thoms,[26] who presented another method which gave him an ash and nitrogen free amorphous strophanthin. Gerrard[27] also stated that the method of Fraser is not clearly published and suggests the removal of the lead with hydrogen sulphide. When we compare the first and second methods of preparation used by Fraser (mentioned before) we observe that in the one first described the lead is removed by passing carbon dioxide for *several hours* through the solution of strophanthin; in the second method, carbon dioxide is passed for *two or three days*. It would seem, therefore, that Fraser himself had trouble in removing the lead. The explanation of this is that there is present a lead salt of acid strophanthin which cannot be decomposed by CO_2. Boehringer & Sons, who prepared the strophanthin used by Feist in his experiments, followed the process of Fraser. By applying the method of Gerrard (which is similar to Fraser's) a brittle amorphous ash and nitrogen-free strophanthin was obtained which contained 7.01% H_2O (dried at 105° in vac.); 0.2983 gm. strophanthin (acc. to Gerrard) dried at 105° in vacuo gave 0.1938 gm. H_2O and 0.5945 gm. CO_2, or in percent:

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[25] Berichte, 1900, 33, p. 2067.

[26] Berichte, 31, p. 271 and 404.

[27] Pharm. Jour. and Trans., 17 (1887), p. 923.

$$C=58.82 \text{ and } H=7.80.$$

Although the investigations are not yet finished, it can be stated that at least two closely related glucosides are present. One, the crystalline Kombe-strophanthin, and another apparently amorphous strophanthin, both yielding the same strophanthidin. The acid amorphous strophanthin obtained from the crystalline glucoside, while showing nearly the same molecular weight, is found, as will appear later, to be only one-third as toxic to frogs as the crystalline strophanthin. The strophanthin investigated by Fraser and by Feist is probably identical with the amorphous preparations of strophanthin prepared by Heffter and Sachs (the analytical data are not taken up in the table) and with the preparation which we made according to the method of Gerrard. The toxicity of this amorphous preparation is almost the same as that of crystalline strophanthin. If this amorphous strophanthin were merely a mixture of crystalline strophanthin, and its acid amorphous modification, this mixture would be less toxic than that of the crystalline variety unless it contained, besides the two, another of greater toxicity than that of the crystalline strophanthin. As no such product is known, it follows that only a minute quantity of the acid derivative is associated with the naturally occurring amorphous strophanthin.

Rhamnose is an easily crystallizable substance and readily yields methylfurfurol. It is thus difficult to understand why Feist was unable to obtain rhamnose in a crystalline state, notwithstanding that he obtained strophantobiose methyl-ether as a crystalline body, and only by distilling this sugar complex with 30% sulphuric acid was methylfurfurol indicated. It is perhaps not superfluous to consider the close relationship of l-mannose and rhamnose as seen by the structural formula (Winther[28] and Hudson[29]).

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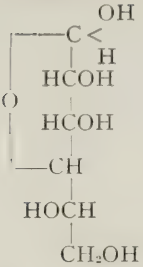
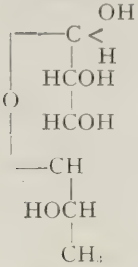
[28] Berichte, 28, p. 3000.

[29] Jour. of Americ. Chem. Soc., 1910, 32, p. 314, and p. 889.

l-mannose



rhamnose



We found further that in large amounts mannose does yield a furfurol derivative. Five gm. d-mannose gave 0.269 gm. blackish-brown phloroglucid. According to Alberda van Ekenstein and Blanksma[30] no furfurol, but β-oxy δ-methylfurfurol is formed by the action of hydrochloric acid on hexoses. Both are precipitated by phloroglucine. Amorphous Kombe strophanthin Merck was also distilled with 12.5% HCl in an endeavor to obtain the phloroglucid of (methyl) furfurol. 1.000 gm. of amorphous strophanthin Merck yielded 0.0568 gm. of a dark-green phloroglucid, which indicated a pentose. According to the formula of Tollens (Journ. f. Landwirtschaft 1900, p. 379):

$$\text{pentose} = (A + 0.0052) \times 1.017$$

gives 6.3% pentose.

Another experiment 1.000 gm. amorphous strophanthin Merck yielded 0.0577 gm. dark-green phloroglucid, or 6.4% pentose.

We have not as yet determined whether this phloroglucid is a mixture of methyl and furforol phloroglucid.

Two molecules of crystalline Strophanthin united by a pentose can explain these observations, but further experiment must decide.

APPENDIX.

While it is known that more than 20 different species of Strophanthus seed may be present on the market[31] there are but three different strophanthins (Kombe-strophanthin, hispidus-strophanthin and gratus-strophanthin or ouabain) of which any chemistry is known. It is a little astonishing that only at the end of a controversy of very well known pharmacognosists and botanists, regarding the question, "What kind of Strophanthus for the Pharmacopœia, in relation to the question, what kind of Strophanthus should be taken for culture?" Arthur Meyer[32] comes to the conclusion that it would be better to investigate first:

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[30] Chemisch Weekblad, 1909, p. 217.

[31] Blondel. Journ. de Pharm. et de Chem., 1888, I, p. 297.

1. Which glucosides are contained in the seed of Strophanthus Kombe and of Strophanthus hispidus? Are they the same or different?

2. Is the clinical action of both drugs and the respective glucosides the same? If not, which drug is to be preferred?

In this paper we have attempted to answer the question relative to the chemistry of Strophanthus Kombe, and Heffter and Sachs have done like service for the Strophanthus hispidus seed.

In connection with the results of Heffter and Sachs the following experiments, which have been made with unidentified Strophanthus hispidus seed, seem to us also worthy of publication. We were able to obtain on the N. Y. market 17 kg. of Strophanthus hispidus seed which could be used for experiment.

3.5 kg. powdered hispidus seed were extracted twice with petroleum ether and gave 950 gm. of a green oil, or 27.1%.

The remaining fat-free powder was percolated with eight times its weight of 70% alcohol. The alcohol was distilled off in vacuo until about 1 liter fluid remained. This fluid was purified with lead subacetate solution and further treated as described before, but no crystals of a glucoside could be obtained. Therefore, we tried to prepare the strophanthidin out of this extract. A large amount of strong alcohol was added, the precipitate filtered and the alcohol of the filtrate removed by distillation in vacuo.

The following method was used: 30 gm. of the thick purified extract was boiled with 150 cc. 1% H_2SO_4 in an Erlenmeyer flask of 500 cc. capacity, until the fluid becomes just turbid. The fluid is now quickly cooled to about 50° and the oily brown drops are broken with a glass rod against the walls of the Erlenmeyer flask, until all is reduced to a yellowish-white crystalline powder. It is important to do this exactly, though it takes about one-half hour to get it powdered in this way. The strophanthidin can be sucked

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[28] Gotz: Berichte der Deutsch. pharm. Gesellsch., 1902, p. 183.
[29] Arthur Meyer: Archiv. d. Pharm., 24?, p. 351.
[30] Hartwich: Apoth. Zeit. 22, p. 101.
[31] Gilg: Bericht der deutsch. pharm. Gesellsch., 18, p. 284.
[32] Arthur Meyer: Archiv. d. Pharm., 246, p. 541.

off on a hardened filter and washed with a little warm water until neutral. The yield of impure strophanthidin was about 10 gm. This strophanthidin was repeatedly recrystallized from boiling alcohol.

After separating the impure strophanthidin the filtrate, when again boiled for 20 minutes and treated in the same way, gave but a small yield of strophanthidin.

Properties. Under the microscope this purified strophanthidin shows the same shape of crystals as Kombe strophanthidin.

Melting point. The air-dried and anhydrous strophanthidin has the same melting point as Kombe strophanthidin. At about 130° C. the air-dried substance softens and at 170° C. it melts. The fused mass resulting from melting and cooling the air-dried product now melts at about 235° C. By drying in vacuo at 110°-115° C., strophanthidin free from water of crystallization could be obtained. Found: 2.9% H_2O calculated for $C_{27}H_{38}O_7+H_2O$, 3.66% H_2O.

Specific rotation. 0.5900 gm. air-dry strophanthidin was dissolved in 25 cc. methyl alcohol.

$$l = 2 \qquad (a)_D = \frac{100a}{l\,c} = 44.07$$

$$a = +2.08^\circ$$

Found for Kombe strophanthidin $(a)D = +44.29$.

Combustion.

I. 0.2345 gm. air-dry strophanthidin gave 0.1721 gm. H_2O and 0.5681 gm. CO_2.

II. 0.2076 gm. air-dry strophanthidin gave 0.1489 gm. H_2O and 0.5027 gm. CO_2.

Or in percent:

	I	II	Calculated for $C_{27}H_{38}O_7+H_2O$
C	66.06	66.04	65.80
H	8.22	8.04	8.20

III. 0.2339 gm. strophanthidin dried at 100°-115° in vacuo gave 0.1654 gm. H_2O and 0.5848 gm. CO_2.

Or in percent:

	III	Calculated for $C_{27}H_{38}O_7$
C	68.17	68.30
H	7.93	8.09

From the conformity of these physical and chemical properties we conclude that this strophanthidin and Kombe strophanthidin are identical.

It was not possible to obtain a crystalline glucoside out of these strophanthus hispidus seed, but the strophanthidin obtained from this glucoside could be identified.

It seems important for a systematic study of the strophanthus seed species and for related species in general to differentiate between their strophanthidins. A quantitative estimation of the strophanthidin not only forms a convenient method for chemical assays, but also their identification is an important factor in determining the species.

PHYSIOLOGICAL.

The physiological activity of crystalline Kombe strophanthin was investigated by the method of Houghton[33] for the standardization of heart tonics of the digitalis series.

This method is based on the minimum lethal dose to frogs. As there is considerable variation in the lethal dose of this series for frogs at different seasons, etc., it becomes necessary, in order to obtain comparable results, to determine, not the toxicity of the sample per se, but its ratio to that of a heart tonic preparation of definite strength (the standard). We are thus independent of variation in the resistance of frogs. The minimum lethal dose of the standard is an *experimental* value, while the heart tonic unit (H. T. U.)[34] is an *adopted* one and will be discussed later in connection with the experiments.

Since Kombe Strophanthus seed is adopted as official by the Pharmacopœias of most countries, it is of real importance that the active principle of Kombe seed be used in comparing the activity of galenical preparations of strophanthus.

In the case of digitalis where a number of differently acting bodies may be present, no one can be used as a basis for comparing the therapeutic value of its galenical preparations. Therefore, since it is impossible at the present time to determine the amount of each of the active bodies, it is better to take a typical heart tonic like crystalline Kombe strophanthin for such comparisons.[35]

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[33] J. Am. Med. Ass., Vol. 31, p. 959, 1898.

[34] Lancet, June 19, 1909. Am. Jr. Pharmacy, Oct., 1909.

In the following experiments frogs (Rana Pipiens) of from ten to thirty grams were used, but with only five grams difference in the weight of frogs for any one comparison. The material was used in such concentration that about 0.5 cc. was needed for an injection. The injections were made by inserting the needle through the mouth into the ventral lymph sac. The frogs were kept in cages in a trough of running water, and at the end of twelve hours the frogs were examined and the results noted.

The toxicity of crystalline Kombe strophanthin is compared with that of some other strophanthins in Table I. The results are calculated for crystalline Kombe strophanthin, at the minimum lethal dose (M. L. D.) of 0.000001 gm. per gm. of frog, which was found to be the mean M. L. D. for the year.

TABLE I.

	M. L. D. per gm. of frogs.
Crystalline Kombe strophanthin (fr. Ident. K.)	0.000001 gm.
Amorphous acid strophanthin (derived fr. above)	0.0000031 gm.
Crystalline gratus strophanthin (Ouabain Merck)	0.00000042 gm.
Amorphous Kombe strophanthin (Merck)	0.00000094 gm.
Amorphous Kombe strophanthin (P. D. & Co.)	0.00000097 gm.
Crystalline Kombe strophanthin (fr. Kombe of trade)	0.00000095 gm.
Amorphous acid strophanthin (derived fr. above)	0.0000031 gm.

It is seen that the crystalline gratus strophanthin (Ouabain Merck) is about twice as toxic as crystalline Kombe strophanthin and the amorphous acid strophanthin from cry. K. strophanthin is practically one-third as toxic. The amorphous Kombe strophanthin, both P. D. & Co. and Merck, and also the crystalline

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[35] It has been suggested that crystalline gratus strophanthin be used for such a standard. However, there are a number of disadvantages in its adoption.

It has never been exactly determined whether the resistance of frogs to the lethal dose of different heart tonics varies differently as the susceptibility of the frog changes with the season, etc. If crystalline Kombe strophanthin is taken as a standard this does not enter into consideration with Strophanthus preparations. With Digitalis we have a large volume of data which shows that this ratio remains very constant, while with gratus Strophanthin very little data is available which bears upon this point.

Gratus Strophanthin or Ouabain, while a nicely crystalline body may contain variable amounts of water of crystallization, and as it does not yield a crystalline strophanthidin the study of its chemistry is under a disadvantage and the identity of the glucoside is to that extent uncertain. Now that we have a crystalline Kombe strophanthin of definite physical and chemical properties that yields in addition a readily crystallizing strophanthidin so that the identity of the glucoside can be reliably determined, there is an advantage in adopting crystalline Kombe strophanthin for such a standard.

Kombe strophanthin from trade Kombe seed, show practically the same toxicity as the crystalline Kombe strophanthin from identified Kombe seed.

The mean lethal dose was determined for crystalline Kombe strophanthin from the average of a large number of tests in comparison with a tincture of Strophanthus, of which the yearly mean lethal dose was known.

The results are found in Table II:

TABLE II.

	Standard Average Tr. of Kombe Strophanthus. (1)			Crystalline Kombe strophanthin, C. P. in solution 70% alc. (2)			
Date.	Dose per gm. of frog in cc.	Result		Dose per gm. of frog in gm.	Result		Corrected for Tr. killing at .000075.
		L.	D.		L.	D.	
1910							
Mar.	.000060	6	0				
10 to	.000065	5	4	.0000008	3	0	.00000085
22	.000070•	2	10	.0000009•	0	[illegible]	.00000097•
Apr.	.000080	5	1	.0000010	4	0	.00000088
8 to	.000085•	1	3	.0000011	1	2	.00000099
11	.000090	0	4	.0000012•	0	3	.00000106•
June	.000065	1	0	.0000008	3	1	.00000086
27, 28	.000070•	0	2	.0000009	4	1	.00000097
	.000075	0	2	.0000010•	0	6	.00000107•
July	.000060	2	0	.0000007	1	0	.0000008
23, 25	.000065•	0	3	.0000008	1	1	.00000092
	.000070	0	4	.0000009•	0	3	.00000104•
Aug.	.000050	5	1	.0000007	5	1	.00000088
to 3	.000055	3	4	.0000008•	1	5	.000001•
	.000060•	1	3	.0000009	0	2	.0000011
Sept.	.000055	2	0	.0000006	1	0	.00000075
6 to	.000060•	1	5	.0000007	3	1	.00000088
19	.000065	0	4	.0000008•	0	2	.0000010•
Oct.	.000070	10	0	.0000009	7	1	.0000009
28, 29	.000075•	3	7	.00000095	5	8	.00000095
				.0000010•	0	10	.0000010•
Nov. 1	.000066	2	0	.0000009	3	0	.00000094
	.000069	1	1	.00000095•	0	4	.00000099•
	.000072•	0	2	.0000010	0	3	.00000104
Dec.	.000070	2	0	.0000009	2	0	.00000084
	.000075	2	0	.0000010	3	1	.00000094
	.000080•	0	2	.0000011•	0	2	.00000103•
1911				(3)			
Mar.	.000080	4	0	.0000010	7	1	.00000084
	.000085	3	1	.0000011	[illegible]	2	.00000092
	.000090•	0	2	.0000012•	0	6	.0000010•
				(4)			
Oct.	.000055	6	2	.00000070	2	0	.00000080
28 to	.000060	4	3	.00000075	5	2	.00000086
Nov. 2	.000065•	2	10	.00000080	1	5	.00000092
	.000070	2	5	.00000085•	0	4	.00000098•
	.000075	0	5	.00000090	2	4	.00000104
				.00000095	1	8	.0000011
1912				(4)			
Apr 25	00006	2	0	.0000007	11	4	.00000075
May 8	00007•	0	2	.0000008	4	8	.00000086
				.0000009•	1	7	.00000097•
				.000001	0	5	.00000107
				(5)			
July	.000055	9	2	.00000075	7	0	.00000080
24	.000060	8	8	.00000080	16	1	.00000086
Aug. 3	000065	5	11	.00000085	7	12	.00000090
	000070•	0	15	.00000080•	0	16	.00000097•
Average	000072			.00000096			.00000100

(1) The standard tincture was kept in the refrigerator.

(2) The crystalline Kombe strophanthin (1 in 1000) in 70% ethyl alcohol was kept at room conditions in amber vials sealed to prevent evaporation. A solution made June 27, 1910, was used in all tests except those made April and June, 1910, unless noted.

(3) Part of these were from solution freshly made up.

(4) These were from solutions freshly made up.

(5) Part of these were from solution freshly made up, and part from solution made up September, 1911, and part from solution of June, 1910 (cf. table of permanency.)

The value .000001 gm. per gm. of frog for the M. L. D. is the mean of the first year's lethal doses for crystalline Kombe strophanthin. If the lethal dose of the standard tincture is taken at .000075 cc. per gm. of frog for the mean M. L. D. of the year, the toxicity of crystalline Kombe strophanthin comes out almost exactly .0000010 gm. per gm. of frog.

The value .000075 cc. for the average lethal dose for this tincture of Kombe Strophanthus is based on the average of a very large number of tests, extending over a number of years. It was the standard previously used for determining the strength of all the heart tonics manufactured by Parke, Davis & Co.

It seems established that the mean M. L. D. for crystalline Kombe strophanthin is .0000010 gm. per gm. of frog (Rana Pipiens of 10 to 30 gm.)

Other methods for determining the physiologic value of heart tonics were compared on this crystalline Kombe strophanthin.

The time necessary for a frog's heart to cease beating has been proposed by Focke[36] as a method for comparing heart tonic preparations.

He endeavors to determine the value of a preparation by the relation $\surd = \dfrac{P}{D.T.}$ where P is the weight of the frog, D is the dose of the material, and T is the time elapsing before complete cessation of the ventricle beat occurs.

The results with crystalline Kombe strophanthin on the heart of frogs are seen in Table III:

TABLE III. Focke Method.

9/23/10 Crystalline Kombe strophanthin.

Dose per gm. of frog.	0.000001 gm.	0.0000015 gm.	0.000002 gm.
Intervals in minutes until ventricles stopped beating.	11 Min. 14 " 13 " 16 " 10 "	13 Min. 14 " 12 " 12 " 10 "	9 Min. 10 " 11 " 10 " 11 "
Average	13 Min.	12 Min.	10 Min.

9/27/10

Dose per gm. of frog.	0.000001 gm.	0.000002 gm.	0.000004 gm.
Intervals in minutes	19 Min. 18 " 15 " 9 " 10 "	16 Min. 13 " 10 " 15 "	10 Min. 10 " 8 " 6 " 10 "
Average	16 Min.	13.5 Min.	9 Min.

The lack of uniformity in the above table is not surprising to anyone who has observed the gradual and erratic cessation of the ventricle beat. The personal factor largely enters on account of there being no sharp end point. After the ventricle has stopped beating, a slight jar or noise will often induce muscular movements of the frog sufficient to force blood into the ventricle, when it will start up and beat again for some time.

We found the method very unsatisfactory and agree with Edmunds and Hale[*] when they say, "We believe that this method allows of greater variations and inaccuracies than any other method we employed."

If the frogs are examined at the end of one hour for complete stoppage of the heart in systole, a more uniform result is obtained, as appears from Table IV:

TABLE IV.

Crystalline Kombe strophanthin (fr. identified seed.)

Dose per gm. of frog	0.0000011 gm.	0.0000012 gm.	0.0000013 gm.
Conditions of heart at 1 hour.	3 beating 0 stopped	6 beating 1 stopped	0 beating 7 stopped

We believe any physiologic method for the assay of heart tonics should have as a basis some definite standard, *e.g.*, Tr. Strophanthus, or better, crystalline strophanthin. The sharper and more definite the end point the more exactly can physiological reactions be compared.

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[*] Bulletin 48, Hygienic Laboratory, p. 16.

Protocol of the toxicity tests of November 1, 1910:

TABLE V.

Cry. K. strophanthin.				Amor. acid strophanthin from cry. K. strophanthin.				Standard Average Tincture.			
Wgt. frog.	Dilution 1 in 50,000		Result.	Wgt. frog.	Dilution 1 in 12,500		Result.	Wgt. frog.	Dilution 1 in 500		Result.
	Dose in gm. per gm.	Full dose			Dose in gm. per gm.	Full dose			Dose in cc. per gm.	Full dose	
13.5	.00000090	.605 cc.	L.	21.5	.0000026	.70 cc.	L.	16.5	.000066	.545 cc.	L.
14.0	.00000090	.63 cc.	L.	17.0	.0000028	.895 cc.	L.	16.5	.000066	.64 cc.	L.
16.0	.00000090	.72 cc.	L.	17.0	.0000028	.595 cc.	D.	17.5	.000069	.60 cc.	L.
14.0	.00000095	.665 cc.	D.	17.5	.000003	.655 cc.	D.	20.5	.000069	.70 cc.	D.
14.0	.00000095	.665 cc.	D.	17.5	.000003	.655 cc.	D.	17.5	.000072	.65 cc.	D.
14.5	.00000095	.69 cc.	D.	18.0	.000003	.675 cc.	D.	20.5	.000072	.74 cc.	D.

In the case of warm blooded animals the toxicity cannot logically be translated into heart action, since death is with them generally due to failure of respiration.

The toxicity for small warm blooded animals, when injected subcutaneously, is given in Table VI:

TABLE VI.

Animals used.	Crystalline Kombe strophanthin. Lethal dose per gram of animal.	Amorphous acid Kombe strophanthin. Lethal dose per gram of animal.	Standard Tr. K. Strophanthus. Lethal dose per gram of animal.
Guinea-pigs	.0000004 gm.	.0000010 gm.	.00095 cc.
Tame mice.......	.000009 gm.	.000025 gm.	.0005 cc.
Tame rats........	.00006 gm.	.00005 gm.	....
Wild rats.........	.000009 gm.	.00008 gm.	.0008 cc.

The guinea-pigs were invariably killed by failure of respiration and the heart was found to be in diastole. The rats and mice generally showed a failure of respiration before the heart action ceased, and the heart stopped in moderate systole.

Crystalline Kombe strophanthin, in aqueous solution, is changed into an amorphous acid strophanthin when boiled. As the toxicity of this amorphous acid strophanthin is only one-third that of the crystalline glucoside, it seemed important to obtain exact figures of this change.

A solution of crystalline Kombe strophanthin in pure water (1 in 2000) was vigorously boiled over a free flame for fifteen minutes, cooled and made up to volume. This tested against the unboiled portion showed a loss of about 15%.

A solution of gratus strophanthin (Ouabain Merck) (1-25,000) in pure water was vigorously boiled over a free flame for fifteen minutes, cooled and made up to volume. This tested against the unboiled portion showed about 10% loss.

As such a change is to be expected in the cold, although, of course, much more slowly, it is important to determine the keeping qualities under different conditions.

A water solution (1 in 2000) of this crystalline Kombe strophanthin was made up and kept in a cork-stoppered flask at room temperature. At the end of two weeks a beautiful growth of mould was present, and an assay showed it to have lost 50 percent of its activity.

Crystalline Kombe strophanthin and Kombe strophanthin (Merck) were then made up (1 to 200) with water containing 4/10% Trikresol and kept at room temperature in sealed containers; no loss appeared with the crystalline glucoside in three months. However, the amorphous preparation of Merck showed a loss of nearly 10 percent within the limits of error.

It was noted that a small amount of alcohol prevented the conversion into the amorphous acid strophanthin, and was taken advantage of in concentrating solutions.

We also found that at room temperature 10 percent alcohol preserves the activity of crystalline Kombe strophanthin.

The marked permanency of the tincture of Strophanthus has long been recognized. It seemed desirable, therefore, to examine this crystalline glucoside from identified Kombe seed in this respect.

Solutions of crystalline Kombe strophanthin (1 in 1000) were made up from time to time with 70% alcohol (and 2 cc. portions sealed in glass containers) and tested on frogs against the old solutions, with the results given in Table VII.

The results of over two years show the really remarkable permanency of such a solution.

No systematic study of the heart action was undertaken. However, a few typical tracings showing the action upon the blood pressure and heart of dogs are given. Plate I shows the action of crystalline Kombe strophanthin, and II that of its amorphous acid modification. These are not given with any view to quantitative comparison. Blood pressure effects and changes in heart action in warm-blooded animals give but an uncertain basis for comparing heart tonics on account of the long duration of the action, so that a subsequent injection may show a cumulative effect. Thus the response to a given preparation cannot be compared with that of a standard on the same animal, and on different animals the variation is too large for practical comparison.

TABLE VII.
Permanency of crystalline Kombe strophanthin in 70% Ethyl Alcohol, kept at room temperature in sealed containers.

Date of Tests	Dose in gm. per gm.	Lived	Died	
March 13 and 15, 1911...	.000,001,0	3	1	Solutions of June 27, 1910.
	.000,001,1	2	1	
	.000.001,2*	0	3	
	.000,001,0	4	0	Solution freshly made from crystals
	.000,001,1	2	1	
	.000,001,2*	0	3	
October 28	.000,000,70	4	0	Computed from standard average tincture.
November 2	.000,000,75	6	2	
	.000,000,80	4	3	
	.000,000,85*	2	10	
	.000,000,70	2	0	Solution freshly made from crystals
	.000,000,75	5	2	
	.000,000,80	1	5	
	.000,000,85*	0	4	
1912	.000,000,80	2	0	Solution of June 27, 1910.
July 24	.000,000,85*	0	2	
	.000,000,90	0	2	
July 25..................	.000,000,80	2	0	Solution of Sept. 25, 1911.
	.000,000,85*	0	2	
	.000,000,90	0	2	
July 26	.000,000,80	3	0	Solution freshly made up.
	.000,000,85*	1	2	
	.000,000,90	0	2	
December 20	.000,000,65	3	1	Solution of Sept. 25, 1911.
	.000,000,70*	1	2	
	.000,000,75	0	3	
December 20............	.000,000,65	3	0	Freshly made up.
	.000,000,70*	0	3	
	.000,000,75	0	2	
December 27	.000,000,60	2	0	Solution of June 27, 1910.
	.000,000,65	2	2	
	.000,000,70*	0	5	
December 27	.000,000,60	2	0	Solution of December 20, 1912.
	.000,000,65*	1	3	
	.000,000,70	0	2	

Tests in connection with the preparation of crystalline Kombe strophanthin showed that strong alcohol precipitated from the tincture of Strophanthus, salts and saponin like bodies which carried down some substances very toxic to frogs and which generally produced a fall in blood pressure and weakened heart action. The dried precipitate killed frogs at .00002 gm. per gm., but upon purifying by reprecipitation the toxicity and depressor action becomes less and less. A typical tracing of the effect on dogs is seen in Plate III.

The amount is so small in Strophanthus preparations that it is probably of no therapeutic importance, although in prepara-

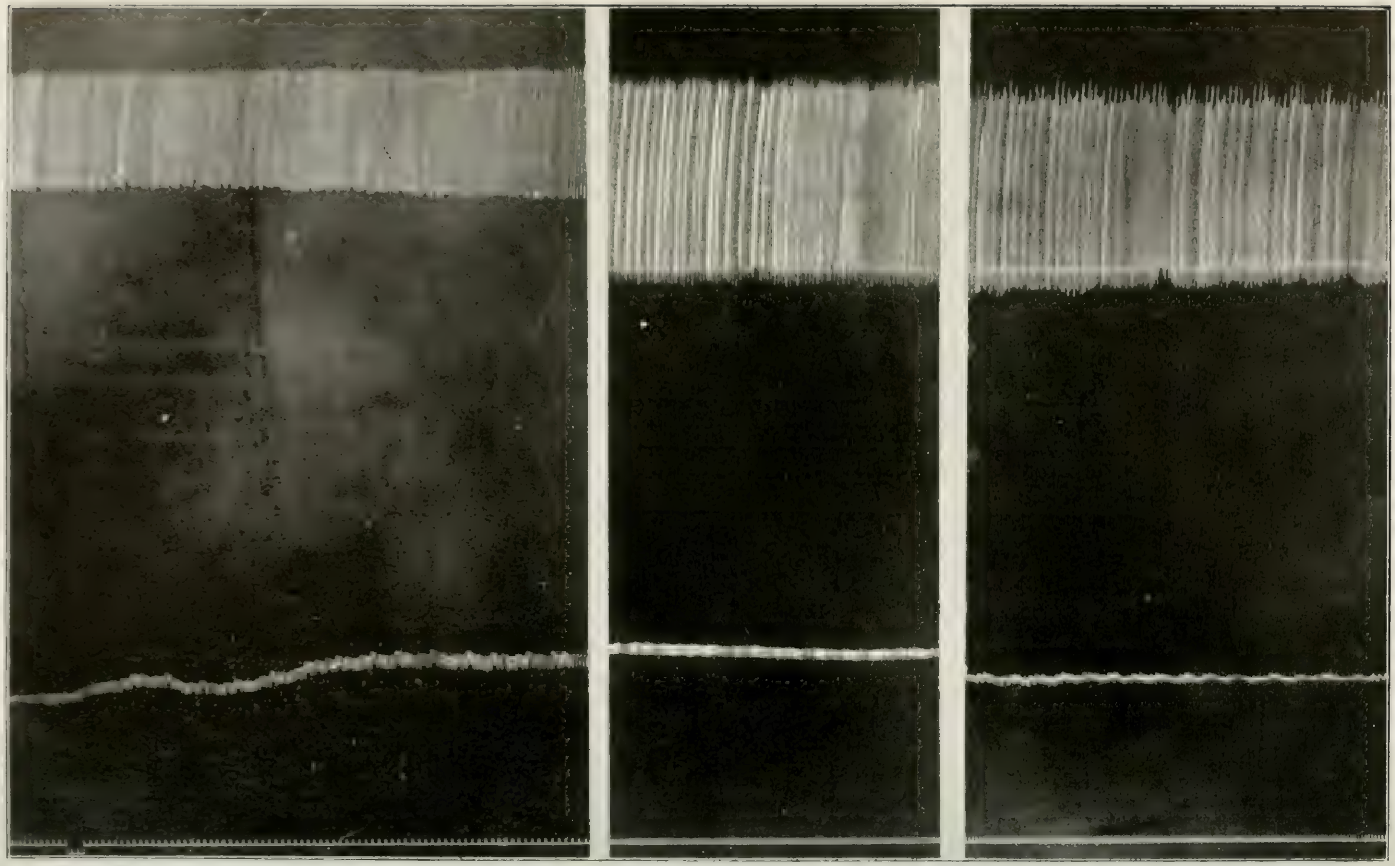

PLATE I.

Plate I shows the effect of the intravenous injection of 0.3 mg. of crystalline Kombe strophanthin on a 12 Kg. dog under chloretone anesthesia and artificial respiration.

Upper tracing is the ventricle beat by direct attachment to the apex and median groove of the heart. Down stroke is systole.

Middle tracing is the carotid blood pressure. Base line time in seconds and signal is at the bottom.

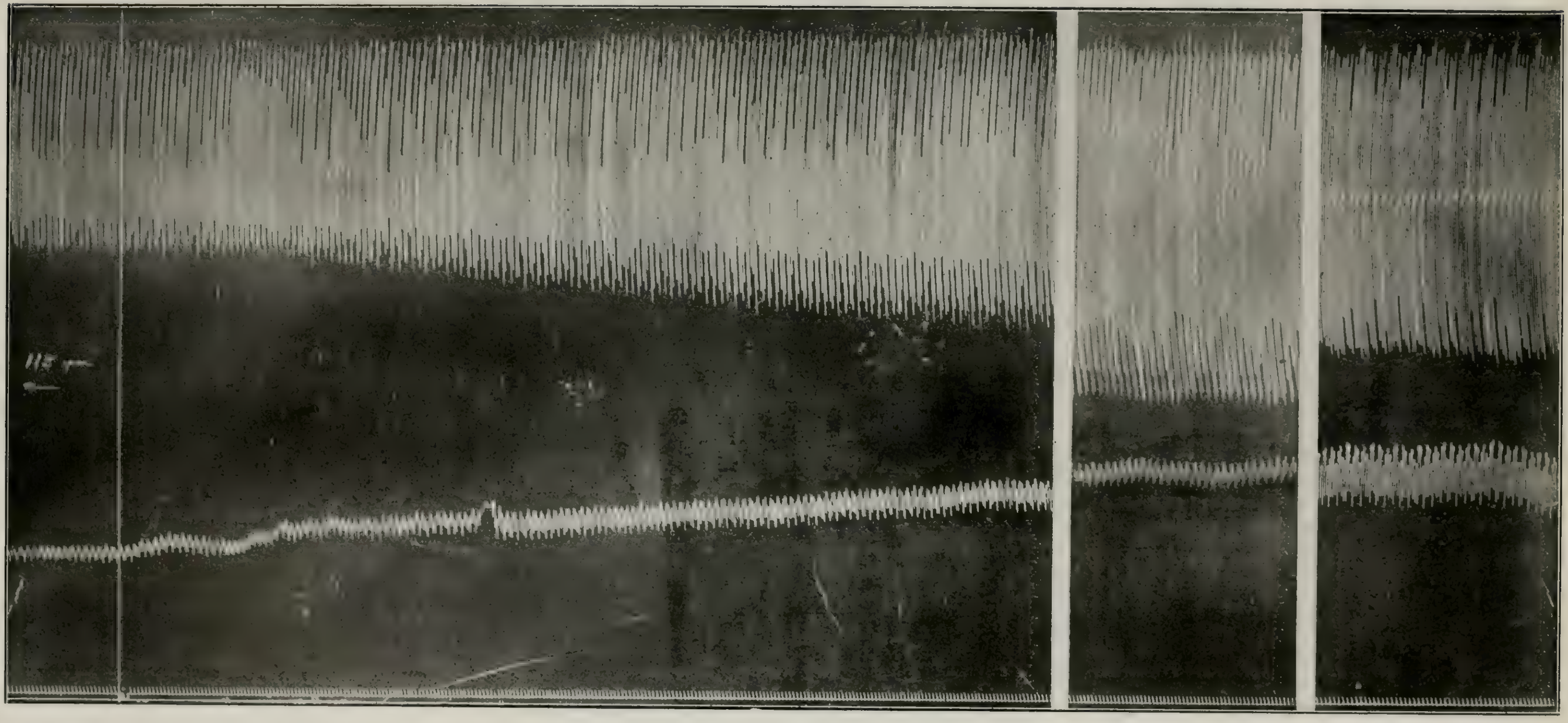

PLATE II.

Plate II shows the effect of the intravenous injection of 1 mg. of amorphous acid strophanthin (derived from crystalline Kombe strophanthin) on a 15 Kg. bitch under chloretone anesthesia and artificial respiration.

Upper tracing is the ventricle beat by direct attachment to the apex and median groove of the heart. Down stroke is systole.

Middle tracing is the carotid blood pressure.

Base line time in seconds and signal is at the bottom.

The second portion of the tracing is one-half hour later and the third portion is two and one-half hours after the injection.

tions of other drugs[39] it may be of importance. Strophanthidin is 1/10 as toxic to frogs as the cryst. Kombe strophanthin from which it is derived, having an M. L. D. of 0.000009 gm. per gm. of frog when the standard killed at .0000009 gm. per gm. of frog.

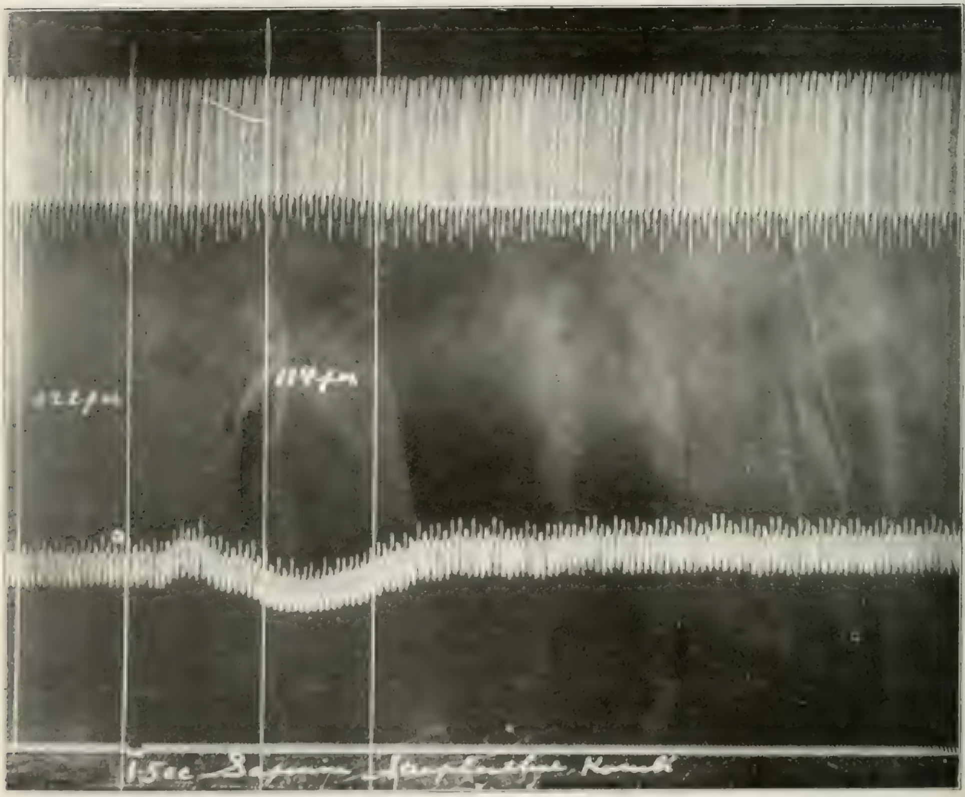

PLATE III.

Plate III shows the effect, on a 13 Kg. dog under chloretone anesthesia and artificial respiration, of the intravenous injection of 1.5 cc. of a solution (1 cc. of which contains 5 mg. of portion precipitated by strong alcohol from tincture of strophanthus and which shows a toxicity to frogs equivalent to .25 mg. per cc. of crystalline Kombe strophanthin.

Upper tracing is ventricle beat by direct attachment to apex and median groove of the heart. Down stroke is systole.

Middle tracing is the carotid blood pressure.

Base line, time in seconds and signal is at the bottom.

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[39] In the case of ergot one of the bodies thrown down with strong alcohol is beta-imidazolethylamine (Histamine), which in this case is considered one of its therapeutically active principles.

Digitalis and very many other drugs show the presence of bodies with very similar properties and they become of importance when the therapeutically active bodies have poor solubility in water or weak alcohol, since infusions and such extracts may have quite different properties from those expected of the drug.

The intravenous injection of strophanthidin has some difficulties on account of its slight solubility in water, but by quickly diluting a weak alcoholic solution a sufficiently fine suspension was obtained for injection. The effect upon the blood pressure and heart action is very similar to that of strophanthin.

DISCUSSION OF PHYSIOLOGICAL RESULTS.

In the hands of the authors, the 12-hour frog method of Houghton (Am. Journ. Pharmacy, Oct., 1909) gave the most accurate and reliable results, and for reasons previously stated seems the most logical one to use in standardizing the digitalis series of heart tonics. Equally logical is the method suggested (same reference) for giving tangible expression to the degree of activity of preparations of the series, namely, to use the M. L. D. of cryst. Kombe strophanthin as the basis for comparison; the ratio of the M. L. D. of each member of the series to that of strophanthin, thus determining the activity. Ten times this M. L. D. was chosen as the Heart Tonic Unit (see also Am. Journ. Pharmacy, March, 1912); therefore, the activity of any preparation is the reciprocal of ten times its M. L. D. adjusted to the average for that drug, by comparison with the M. L. D. of the standard (strophanthin). Whatever method is employed, however, it is necessary to compare the activity of an unknown preparation with that of a known or standard. This comparison can be expressed only in terms of a definite amount of the standard, and it is the logical procedure to speak of this quantity as a heart tonic unit (H. T. U.) What amount shall be adopted is unimportant. For our own work we have adopted the standard heart tonic unit (H. T. U.) as 0.00001 gm. of crystalline Kombe strophanthin as suggested above. This is ten times the minimum lethal dose for frogs (Rana Pipiens, 10 to 30 gm.).

The marked stability of alcoholic solutions of crystalline Kombe strophanthin is a very important point in connection with the use of this crystalline glucoside as a standard for determining the activity of galenical preparations, not only of strophanthus seed, but of other heart tonics.

The comparison of the physiological and chemical properties of crystalline and amorphous acid strophanthins shows that the molecular rearrangement brought about by the introduction of one molecule of water has markedly altered the physiological activity.

Other cases are known in which the addition of a molecule of water is accompanied by marked changes in physiological activity, as inactive ergotinin into active ergotoxin.

In the case of the conversion of crystalline Kombe strophanthin to the amorphous acid strophanthin there has been the change of a lactone group into its acid and alcoholic portions, and accompanying this change a loss of two-thirds its toxicity to frogs.

The character of the blood pressure changes and heart action does not appear to be altered by the opening of one lacton ring formation, only a loss of activity is observed. It must be remarked that the loss of activity as observed with dogs was not as great as indicated by the toxicity tests on frogs and guinea-pigs, and also the toxicity to rats and mice show that the relation one to three is not true in all respects for the crystalline Kombe strophanthin and the amorphous acid Kombe strophanthin.

RESUME.

The seeds of Strophanthus Kombe Oliv. contain two strophanthins: a crystalline glucoside of the formula $C_{40}H_{56}O_{15}+3H_2O$ and a closely related amorphous strophanthin of apparently twice the molecular weight. By the action of water on crystalline Kombe Strophanthin there is formed a monobasic acid strophanthin or a mixture e. g. of a monobasic acid, a dibasic acid and the original crystalline strophanthin. These three strophanthins, crystalline, its acid derivative and amorphous Kombe Strophanthin, when split by dilute acids give strophanthidin of the formula $C_{27}H_{38}O_7+H_2O$. This strophanthidin is identical with the strophanthidin described by Feist and by Heffter and Sachs.

Crystalline Kombe strophanthin contains neither a pentose nor a methyl pentose (rhamnose). Amorphous Kombe strophanthin apparently contains a pentose. The crystalline Kombe strophanthin prepared by Arnaud is doubtless identical with that prepared by us, but Arnaud was at fault in considering as a hydrate a new chemical derivative, which we have spoken of as amorphous acid strophanthin.

The results of Kohn and Kulisch show a marked conformity with those of Arnaud and of our own upon amorphous acid strophanthin in everything except the data upon strophanthidin. It seems probable that the method of cleavage and purification accounts for the different strophanthidin.

Crystalline Kombe strophanthin apparently undergoes the following cleavage when heated with dilute acids:

$$C_{40}H_{56}O_{15}+4H_2O=C_{27}H_{38}O_7+C_{12}H_{22}O_{11}+CH_4O.$$

Crys. K. strophanthin. strophanthidin disaccharide methyl-alcohol.

Notwithstanding the uncertainty as to the purity of amorphous substances we have shown that a strophanthin different from crystalline Kombe strophanthin is present in identified Kombe seed. Heffter and Sachs have shown that identified hispidus seeds do not contain a crystalline strophanthin, but an amorphous one which is identical or closely related to the amorphous strophanthin from Kombe.

Both crystalline Kombe strophanthin and amorphous acid strophanthin show the typical heart tonic response, diminished rate and increased amplitude of the heart beat, accompanied by a small rise in blood pressure.

The activity of the amorphous acid strophanthin is less than that of the crystalline strophanthin. By the frog method of Houghton, the activity of these strophanthins is in the ratio one to three.

It is very interesting to note that this loss of activity is associated with the loss of one lacton group.

We believe this crystalline Kombe strophanthin as the definite active constituent contained in Strophanthus Kombe Seed, U. S. P., should be adopted as the standard by which the value of the various preparations of the drug should be measured.

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73899

A COMPARATIVE STUDY OF ANTIGENS FOR THE WASSERMANN REACTION.*

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HISTORICAL REVIEW.

In 1901, Bordet and Gengou published the method for complement fixation, which was designed for the purpose of detecting antigens; that is, such bodies as are capable of producing antibodies, as well as a definite group of serum substances, probably amboceptors, the nature of which is not yet determined. It is possible by this method to determine, by the use of a known antigen, whether a certain serum contains the specific amboceptor, or reversely, one may determine by means of a known serum whether the substance used as antigen contains the antigen bodies. Neisser and Sachs proposed the use of this method for the differentiation of albumin. Wassermann, Neisser and Brück were able to demonstrate, in the serum of monkeys which had been previously treated with extracts of syphilitic organs, the presence of substances which gave complement fixation with syphilitic extracts. Detre, as well as Wassermann, Neisser, Brück and Schucht, found the same substances in the serum of syphilitic patients. Their early experiments, however, gave very inconsistent results. Citron was the first to recognize that the amounts of antigen and serum employed by the former authors had been too small and that the influence of the specific medication on the reaction was unknown to them.

Porges and Meier, von Landsteiner, Müller and Pötzl and later Yamanouchi and others pointed out independently that a substance could be obtained by extracting syphilitic and normal human liver as well as other organs with alcohol, which, when mixed with the serum of syphilitics, gave rise to complement fixation, and which to a certain degree could replace the syphilitic antigen. Porges and Meier, as well as Landsteiner, incline to the view that the alcohol-soluble substance is a lipoid closely related to lecithin, while Levaditi and Yamanouchi ascribe less significance to the lecithin and consider the bile salts as the important substances. The complement-fixation tests carried on by Wassermann and Citron with various colloidal substances such as glycogen, albumoses, peptone, lecithin, oil and gelatin, as well as the experiments of Landsteiner and Stanovic with other colloids, led Citron and Wassermann to the assumption that the cause of the complement fixation is a chemico-physical change of the molecule due to its entering into combination with some other substance. The experiments of Seligmann also prove that in colloidal reaction with or without precipitation complement may be absorbed. The disappearance of the complement is not due to the molecular condition as such, but is due to the changed condition of the colloids. While this does not offer any explanation of the specific immunity reaction, it shows that aside from the immunity reaction there coexist other definite chemical reactions which cause the disappearance of the complement. This in no way alters the great value of the complement-fixation test for the biologic diagnosis of infectious diseases, for it is the special property of immune serums to influence to a greater degree that colloid which is their antigen.

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* Read in the Section on Dermatology of the American Medical Association, at the Sixty-Fourth Annual Session, held at Minneapolis, June, 1913.

These findings induced Wassermann to separate the complement fixation in syphilis from the generally accepted antigen-antibody hypothesis for other infectious diseases, for he assumed that all antigens must be albumins or derivatives of albumins, without considering that some lipoids or lipoid-albumin combinations might be antigens also. The experiments of Metchnikoff and those of Deyke Pasha and Reschad Bey point to the fact that it is possible to stimulate the organism to the formation of reacting agents, similar to antibodies, by the injection of fat-like substances. The cobra-lecithin experiments should also be mentioned in this connection. It was pointed out by Citron that the injection of animal and other poisons with lecithin causes the formation of toxolipoids which may act as antigen, that is, produce antibodies. Such antibodies against the syphilitic toxolipoids may have a lipoidophil group which is brought out in the admixture with lecithin.

The fact that the serums of luetic patients are rich in ferments capable of splitting certain fats and lipoids and the presence of such ferments to a lesser degree in the serums of patients suffering from other infectious diseases, such as leprosy, tuberculosis, scarlet fever, etc., may be the reason why such serums react with syphilitic antigen. Thus it is possible that in the Wassermann reaction we are dealing with several distinct complement-fixation reactions of entirely different character, one of which may be specific.

In spite of the fact that the Wassermann reaction is as yet unexplained, it is widely used for the diagnosis of syphilis. The aqueous extracts of the syphilitic liver and spleen were, on account of their instability, displaced by alcoholic extracts of these syphilitic organs. The difficulty of obtaining syphilitic fetal tissue also had its influence in stimulating the search for some substitute as antigen. More recently acetone has been employed as extractive, because it is supposed that such extracts contain substances which approximate the syphilitic antigen more closely, and thus permit of a more delicate reading of the test. It does not fall within the scope of this paper to review the extensive literature dealing with the various antigens employed by the experimenters in this field of research. The extracts employed have been either specific, such as were obtained from syphilitic tissues by the use of water, alcohol, or acetone as extractives, or they have been non-specific extracts made from heart muscle, liver and brain of guinea-pigs and cattle. Aside from these extracts, combinations of chemical substances, such as Schurmann's and those given by Sachs and Rondim, have been employed as antigens in this reaction. From this it appears that normal tissue contains substances, possibly to a lesser degree, as has been shown by Much, which have the power to absorb the complement.

Aside from the use of artificial antigens, there have been other modifications of the Wassermann reaction; however, none of these modifications have as yet increased the clinical value of the test, nor has any attempt to simplify the method been suggested which does not at the same time sacrifice considerably its accuracy.

In the course of the inoculation experiments of four strains of the *Spirochæto pallida,* which have been carried on in rabbits at the Research Laboratory of Parke, Davis & Co., it seemed that the white, glistening gumma, which contains practically nothing but spirochetes and the products of their metabolism, might offer material for the preparation of antigen for the Wassermann reaction which would possess more of the specific antigen bodies than any other antigen yet used for such purposes. The antigen thus prepared was made from lesions caused by the four strains of the organisms. We had, therefore, a specific and to some degree polyvalent antigen. The four strains of *pallida* have been transplanted now for over a year, and the lesions which these organisms produce are free from secondary infection. No antigen was prepared from cultures of the organism, since the culture does not afford a sufficient amount nor could the mediums in which the *pallidæ* were growing be separated from them. It seemed that the extract of organisms and culture mediums would afford us no more specific antigen than had been employed by others thus far. The syphilitic gumma frequently replacing one-half and more of the testis seemed better suited for this purpose, since all the non-infected tissue could be trimmed away, and only the grayish-white gumma used. The term "gumma" is here used not in its strictest sense. The lesion produced by the action of the *pallida* in the gland proper is one of clearly circumscribed mucoid degeneration. Superficial chancres were not employed since these are usually contaminated with other bacteria.

The antigens were prepared as follows: The lesions were carefully freed from the tissue not involved, ground up fine and weighed. The material was then frozen in an ice and salt mixture, and dried *in vacuo* over sulphuric acid. When completely dry, it was placed in physiologic salt solution to which 0.3 per cent. trikresol had been added. The ratio of tissue to salt solution is 1:4. This tissue suspension was shaken for twenty-four hours and placed in a sterile amber glass bottle in the refrigerator for three days to allow the tissue to settle. This was our aqueous antigen.

The alcoholic antigen was prepared in the same way that the regular stock syphilitic fetal liver antigen is prepared.

The acetone extract was prepared by placing the ground-up and dried tissue into Merck's C. P. acetone, taking for every gram of tissue 20 c.c. of acetone. This was allowed to stand in a dark bottle at room temperature for eight days, filtered and evaporated at 37° C. (98.6° F.).

The residue was taken up with C. P. methyl alcohol, one hundred parts of the methyl alcohol being used for every gram of residue. The acetone residue contains a substance which is not soluble in methyl alcohol. The methyl-alcohol-soluble extract is admixed with ten times its volume of physiologic salt solution for the test.

These three extracts of gumma of rabbits were tested against the alcoholic extracts of syphilitic fetal liver, and in our last experiments, the acetone-insoluble portion of beef heart.

Practically every antigen has the power to bind some complement. And the reason for the diversity of results obtained by some investigators on one and the same serum does not lie so much in the method employed as in the proper titration of the extract employed as antigen.

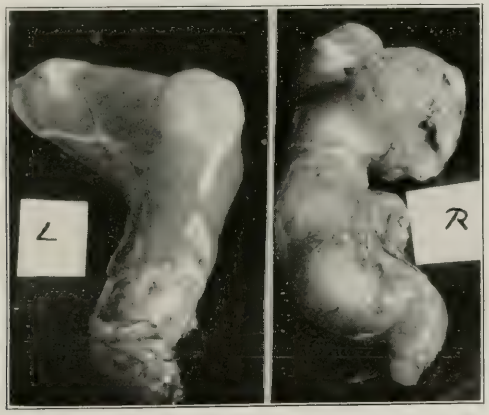

Fig. 1.—Syphilitic gumma of testis of rabbit from which antigen was prepared.

The value of a certain extract is usually determined by a preliminary experiment, usually according to Wassermann, in which a unit of the antigen gives complete fixation when admixed with a unit of luetic serum, while normal serums give a negative reaction. Any antigen fulfilling these requirements is considered satisfactory for this reaction.

If increasing doses of an antigen are tested for their activity in the seroreaction in syphilis, then it is shown that very small amounts will not bind the complement even in cases of florid syphilis. As the dose of the extract is increased, the fixation of the complement occurs with greater frequency, so that serums of latent syphilitics also react. If the amount is increased still more, the serums of patients not syphilitic will give positive reactions. And if the dose of the extract is carried still higher, we find that serums of normal healthy individuals give the reaction. The amount of antigen is then able to bind all the complement. It thus becomes evident that only such amounts of antigen should be employed in syphilis as will bind the complement only with luetic serums. Thus the Wassermann reaction is specific only within certain limits of the doses of antigen. For this reason the extracts were tested for their power to bind complement.

Increasing amounts of the extract are placed with the amount of complement usually employed in the seroreaction in the incubator, then amboceptor and sheep blood corpuscles are added. For the test only one-half of that amount is used, which clearly indicates fixation (*Eigenhemmung*).

DETERMINATION OF THE SENSITIVENESS.

In the serodiagnosis of syphilis, it is not so much a question of obtaining fixation of complement with undoubted cases of syphilis, as of determining a fixation of complement in the doubtful cases, which determine for or against syphilis. Complete binding of the complement is practically uniform with all extracts, but the cases demanding especial attention are those which show only partial complement fixation. Such cases may be overlooked by an observer who does not work with a sensitive antigen, for which reason it is not advisable to take the serum giving a strongly luetic reaction, for a positive control, but one which lies on the border-line, that is, serums that give a weak positive reaction, such as latent luetics, and those having sclerosis. Such serums are tested with varying amounts of extract according to the usual method, beginning with minute dilutions of antigen to the largest permissible.

DETERMINATION OF THE SPECIFICITY.

It is evident that for this purpose not only serums of normal individuals should be employed, but also serums which, from experience, at times give a positive Wassermann reaction. It has

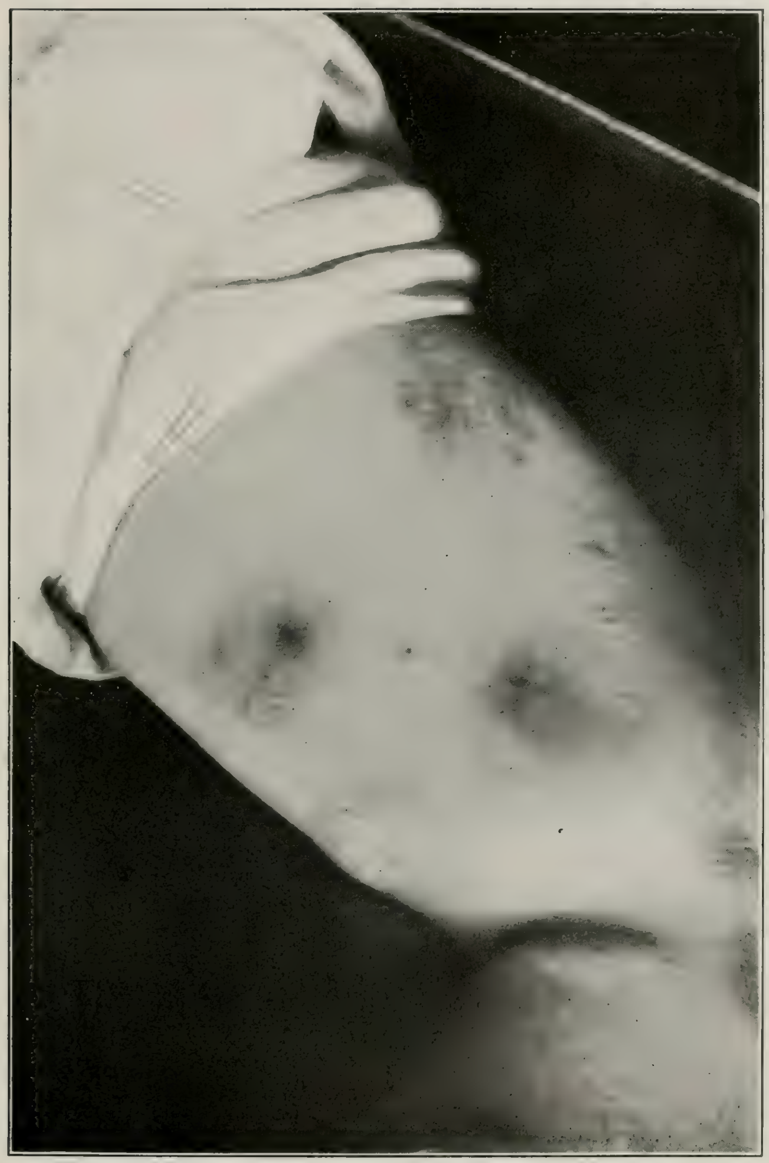

Fig. 2.—Luetin reaction obtained from gumma in rabbits used for the preparation of antigen. Control in center.

already been pointed out that in such cases we may not be dealing with the same biologic process which takes place in the serum of a luetic, but with a non-specific process which simulates the specific reaction in syphilis. Many serums from cases of various general diseases contain substances which inhibit hemolysis. If to such serums antigen is added which also contains bodies capable of binding complement, we are able to observe a summation of these two binding substances which absorb all the complement.

The specificity of the antigen used was determined by the luetin reaction, in so far as this reaction may be called specific. Extract of the gumma gave positive reaction in tertiary cases of this disease.

These comparisons of the antigens of gumma cover only the preliminary stages of the work we have begun. The antigens prepared from the several strains of the spirochetes are more active and for this reason had to be diluted from ten to twenty times more than the ordinary antigens prepared from syphilitic fetal organs or normal tissues. This high dilution of the antigen minimizes the physical influence, which strong alcoholic antigens exert on the serums to be tested. The antigens when admixed with physiologic salt solution were practically clear, differing in this respect from other antigens which in dilution show milky white turbidity. Another difference noticed between the rabbit gumma antigen and other antigens is the more delicate reading of the results of the reaction. The cases of doubtful clinical diagnosis are more clearly brought out by these antigens.

The sensitiveness of these antigens to luetic serums led us to test them against serums from leprosy and scarlet fever. Through the kindness of Dr. Guy L. Kiefer, health officer of Detroit, we were able to obtain serums from scarlet fever patients while the disease was in its eruptive stage and at different periods following this stage. The leprosy serums included were obtained through the courtesy of Dr. James MacFarlane Winfield of Brooklyn, to whom we desire to express our thanks.

The syphilitic lesions in rabbits permitted us to obtain antigens which to a degree are more specific than the antigens generally employed, since these gumma contain the spirochetes in large numbers free from contamination. At the same time we were reasonably certain that our antigens were free from the bile salts which possibly are present in the antigens made from fetal syphilitic livers.

The fact that Schwartz and McNeil were able to improve the fixation of complement in gonorrhea by combining twelve strains of the gonococci of their antigen led us to combine the four strains of the *pallida* for the antigens we employed. By so doing, we increased the efficiency of the antigens. The reaction was negative, however, in the serums of patients having just passed the eruptive stage. The serums of leprosy patients were also positive, yet to a less degree than the luetic controls. How far this reaction is due to the leprosy *per se,* it was impossible for us to determine.

SUMMARY AND CONCLUSIONS.

1. The use of syphilitic gumma in the testis of rabbits yields a more specific antigen than any obtained from other syphilitic or normal tissue.

2. The combination of a number of strains, giving a polyvalent antigen, increased the value of the antigen, permitting the diagnosis of clinically doubtful cases.

3. Aqueous antigens lose their strength in about one month while those prepared with acetone and alcohol are stable.

From our data, we incline to the view that in the Wassermann reaction of syphilis, we may be dealing with three distinct reactions. First, we deal with the antibodies produced against the causative organism—if such antibodies are produced; next, we are dealing with substances, possibly antibodies, directed against bacterial lipoids, and, finally, with a group of substances, autolytic in nature, which the tissues of the body evolve against the toxic substances resulting from the influence of the *Spirochœta pallida* on the tissues.

ABSTRACT OF DISCUSSION.

Dr. Howard Fox, New York: The antigen is the all-important factor in the Wasserman reaction, but the attempts that have thus far been made to standardize our preparations have not been particularly successful. It would be a great step in advance if we could find a standard antigen which every one could use, as in that way there would be fewer divergent results from different laboratories. During the past year I have used the alcoholic extract of normal heart, titrating the complement and using decreasing amounts of the serum of the patient. By this method I have obtained better results than I did formerly with the alcoholic extract of fetal liver.

Dr. J. Frank Waugh, Chicago: There is one other antigen which I do not think has been mentioned. Noguchi has done a great deal of work on the serodiagnosis of syphilis, and in one of his articles on the subject he mentions an insoluble acetone antigen. Dr. Harris gave me some soluble acetone antigen, and on testing it I found it very efficient. The acetone extract gives a stronger reaction in some cases than the alcoholic extract. In the last year I have tried and compared both the alcoholic and the aqueous extracts with the acetone extract and have found the latter just as efficient. I agree with Dr. Fox regarding the importance of a strong, standard antigen.

In the *Deutsche medizinische Wochenschrift* (Dec. 5, 1912), O. Stiner reported his experiences with the use of acetone extracts. He reports that in some cases of positive lues, in which alcoholic extracts gave negative results, positive reactions are secured with an acetone-liver extract.

Dr. M. L. Heidingsfeld, Cincinnati: I take exception to Dr. Varney's statement that the gonococcus complement-fixation test is more reliable than the Wassermann, in order to spare the Wassermann too much undeserved disrepute. Theoretically, the gonococcus test should be more reliable than the Wassermann, but experience has shown that the Wassermann is far the more reliable of the two. The Wassermann never errs on the unsafe side, and is a reliable procedure if the details are carefully and properly carried out. As Wassermann has pointed out, there is a fundamental reason for every step of the reaction. Some of the modifications may be more delicate, but they are at the same time the less reliable.

Dr. Rudolph Buhman, St. Louis: Did Drs. Varney and Baeslack use a watery or an alcoholic preparation as an antigen?

Dr. H. R. Varney, Detroit: I thoroughly agree with Dr. Fox that what we are searching for is a standard antigen, and that when it is found we shall have more uniform laboratory reports. Regarding the preparation of the antigen, the aqueous, acetone and alcoholic were used and checked up in series.

Regarding my estimate of the comparative accuracy of the Wassermann and the gonococcus fixation tests, which Dr. Heidingsfield criticized, I would say that, so far as I have been able to follow the laboratory records, am able to assert that by the fixation tests it is possible to pick out the diplococcus of Neisser from a large family of similar organisms, which is proved clinically, while with the Wassermann test we cannot with our present knowledge positively exclude other diseases, as tuberculosis, scarlet fever or leprosy.

Dr. F. W. Baeslack, Detroit: The delicacy of the test depends largely on the titration of the antigen. We may titrate for its delicacy and sensitiveness, and it is only when tested against weak syphilitic cases that a real idea of the value of the antigen is obtained. It is in the careful titration of the antigen that the secret lies, and what we are searching for is a reliable antigen.

73909

THE TREATMENT OF TETANUS.*

Charles T. McClintock and Willard H. Hutchings.

(From the Research Laboratory of Parke, Davis & Company, Detroit, Michigan.)

This research was undertaken with the object of determining as far as is possible, with animals, the relative value of the various methods of treatment of tetanus.

After a trial of a number of laboratory animals, sheep were selected for the reason that they are susceptible to tetanus, large, and easily handled, they could be bled readily, and because it was possible to infect them in a manner approaching clinical conditions. A 14-day broth culture of a virulent strain of tetanus containing large numbers of bacilli and spores was heated at 65° C. for 20 minutes, for the purpose of destroying the toxin.

In order to be sure of this a part of the material was passed through a Berkefeld and then through a Pasteur filter. One-cubic-centimeter doses of the filtrate thus obtained, when injected subcutaneously into 350-gm. guinea-pigs, failed to produce tetanus. Small pieces of wood were macerated in this toxin-free, spore-containing fluid for two weeks, at the end of which time they were removed and dried.

The method of inoculation was as follows: A sheep with a long tail having been selected, an incision one centimeter in length was made through the skin as near the tip as possible. A pocket was now made by blunt dissection. Into this one piece of infected wood was placed and the wound closed by cat-gut sutures. With the onset of symptoms of tetanus the tail was amputated 20 cm. above the point of inoculation. No anesthetic was used and antisepsis was observed in all operations.

In order to estimate the amount of toxin present in the blood of the sheep two cubic centimeters were removed at 24-hour intervals and injected into the right hind leg of a 350-gm. guinea-pig. In this way a series of animals was obtained which passed from the normal through the various stages of local and general tetanus to the death of animals. Thus a reasonably

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accurate idea of the amount of toxin present in the blood at the time could be obtained.

For the purpose of studying the effects of amputation, without any other treatment, a series of experiments was undertaken by one of us.* Fifty guinea-pigs, 30 dogs, and 10 sheep were used. Immediately on the appearance of symptoms the affected members were amputated. No other treatment was used. Among the conclusions drawn was the following: "Amputation immediately following the first appearance of symptoms affected in no way the progress of the disease. Animals which were amputated died in the same time and with the same symptoms as those which were not amputated."

From these results it is reasonable to conclude, therefore, that any benefit obtained by treatment is the result of this treatment and not of amputation.

Having established this point, we undertook the study of the various methods of treatment, as follows: antitetanic serum, carbolic acid method of Bacelli, magnesium sulfate, chloretone† alone and in combination with serum.

Six sheep were used in each series, five being treated while one was used as control. In every series the control animal died with practically identical symptoms in each case.

TABLE 1.

CONTROL SHEEP.

SHEEP No.	Series.	Inoculated.	Symptoms.	Amputated.	Results.
14..........	Carbolic acid...........	Oct. 29	Nov. 5	Nov. 5	Died Nov. 11
112..........	Magnesium sulphate..	March 26	April 1	April 4	" April 6
301..........	†Chloretone............	July 1	July 8	July 8	" July 12
163..........	†Chloretone and serum..	" 13	" 20	" 20	" " 24
274..........	Serum..................	Dec. 13	Dec. 13	Dec. 13	" Dec. 15

A SAMPLE PROTOCOL.

Sheep 14, weight 32.6 kilos, control, inoculated as described on October 29, 1909. Sheep appeared normal until 7 days later, when there was noticed a slight stiffening of the front legs when the sheep attempted to run. The neck was held rigid with the nose slightly extended. Eating and drinking were normal. The tail was at once amputated 20 cm. above point of inoculation under antiseptic precautions and the wound closed with cat-gut. Healing of the wound was uneventful.

Eighth day: Symptoms were more marked; the sheep walked with

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*[illegible], *[illegible] Festschrift*, p. 601, Leipzig, 1907.
†Chlorobutanol.

some difficulty and fell to knees when attempting to run. Neck stiffer, ate and drank well, chewed cud, and had no difficulty in swallowing.

Ninth day: The sheep remained lying down, but could get up with difficulty. Stiffness pronounced. Ate and drank little. Did not chew cud.

Tenth day: Could not get up alone and when placed on feet could take only a few steps without falling. Ate nothing and swallowed water with difficulty. Stiffness of whole body pronounced.

Eleventh day: Lying on side with head hyper-extended, legs extended and perfectly rigid, could not stand.

Twelfth day: About the same as on the previous day; a few clonic spasms were seen.

Thirteenth day: Clonic spasms frequent but not severe, lasting about 30 seconds. Died during the afternoon.

The following table shows the result of the injection of this sheep's blood into the guinea-pigs.

TABLE 2.

Pig.	Weight gm.	Inoculated with 2 c.c. on	Symptoms.	Date.	Result.
1....................	250	Oct. 30	—	....	Recovered.
2....................	305	" 31	—	....	"
3....................	275	Nov. 1	+	Nov. 5	"
4....................	290	" 2	+	" 5	"
5....................	308	" 3	+	" 5	Died.
6....................	310	" 4	+	" 6	"
7....................	315	" 5	+	" 6	"
8....................	315	" 6	+	" 7	Recovered.
9....................	305	" 7	+	" 10	"
10....................	335	" 8	+	" 10	"
11....................	280	" 9	+	" 11	"
12....................	325	" 10	+	" 13	"
13....................	330	" 11	+	" 14	"

From Table 2 it will be seen that measurable amounts of toxin appeared in the blood on the third day, that the toxin was at its maximum from the fifth to the eighth, and persisted until death on the thirteenth day, but that after the eighth day the amount present was small. On the other hand, the clinical symptoms were not discoverable until the seventh day and reached what might be termed a maximum on the eleventh day. Had this train of symptoms appeared in the ordinary case of tetanus in human beings it is probable that the patient would not have come under observation before the eighth day and a diagnosis would not have been reached until the ninth or even the tenth day. From the table one would be inclined to believe that the decrease of toxin in the circulation after the eighth day was a result of the amputation, removal of the source of supply, but that this is not true we have been repeatedly shown. We have found that the amount of demonstrable toxin in the blood of animals having tetanus, with and without amputation, runs a course practically identical with that shown in the table.

SERUM.

Six sheep were used, five treated with intravenous injection of 2,000 units of antitetanic serum every 24 hours, one control. The control died (274). Of those treated four died and one recovered. The protocol of the one that recovered is as follows:

Sheep 108, weight 55.5 kilos.

December 6: Inoculated in tail as usual.

Seventh day: Showed marked stiffness of head and neck. Some stiffness in forelegs, otherwise normal. Tail amputated 20 cm. above point of inoculation. 2,000 units serum injected into left jugular vein.

Eighth day: Somewhat stiffer than day before. Walked with difficulty. While being prepared for bleeding and injection, had several slight clonic spasms and a peculiar "thrill" which is characteristic of tetanus in guinea-pigs. Ate and drank little. 2,000 units serum injected into left jugular vein.

Ninth day: No worse than on previous day. 2,000 units serum injected.

Tenth day: About the same except that he ate and drank better. 2,000 units serum injected.

Eleventh to sixteenth day: Condition remained stationary. 2 000 units of serum were given on each of these days, when its use was discontinued. The sheep developed chronic tetanus and was not completely recovered until February 1.

The total amount of serum given was 20,000 units.

TABLE 3.

RESULTS OF INOCULATION WITH BLOOD OF SHEEP 108.

PIG.	Weight gm.	Inoculated with 2 c c. on	Symptoms.	Date.	Result.
1....................	350	Dec. 6	—	....	....
2....................	"	" 7	—	....	....
3....................	"	" 8	—	....	....
4....................	"	" 9	—	....	....
5....................	"	" 10	+	Dec. 13	Recovered.
6....................	"	" 11	+	" 13	Died.
7....................	"	" 12	+	" 14	"
8....................	"	" 13	+	" 16	Recovered.
9....................	"	" 14	—	....	....
10....................	"	" 15	—	....	....
11....................	"	" 16	—	....	....
12....................	"	" 17	—	....	....
13....................	"	" 18	—	....	....

The protocol of the sheep which died follows:

Sheep 277, weight 40 kilos.

December 6: Inoculated as usual.

Eighth day: Showed slight stiffness in forelegs, head was slightly extended, otherwise normal. Tail amputated. 2,000 units of serum injected intravenously.

Ninth day: Stiffness increased, drank well but ate little. 2,000 units of serum injected intravenously.

Tenth day: Stiffness very marked, unable to walk but could stand. Refused all food and could not swallow water. Rapid breathing. 2,000 units of serum injected intravenously.

Eleventh day: Very little change from previous day. 2,000 units of serum injected intravenously. Died during the night.

TABLE 4.

RESULTS OF INJECTIONS WITH BLOOD FROM SHEEP 277.

PIG.	Weight gm.	Inoculated with 2 c.c. on	Symptoms.	Date.	Results.
1	320	Dec. 5	—	....	....
2	300	" 7	—	....	....
3	335	" 8	—	....	....
4	300	" 9	—	....	....
5	250	" 10	+	Dec. 14	Recovered.
6	255	" 11	+	" 13	Died of tetanus.
7	330	" 12	+	" 14	" Dec. 17
8	330	" 13	+	" 14	" " 18
9	325	" 14	+	" 15	" " 18
10	325	" 15	—	....	....
11	325	" 16	—		
12	326	" 17	—	....	....

TABLE 5.

SHEEP TREATED WITH SERUM ALONE.

SHEEP.	Inoculated.	Symptoms.	Amputated.	Treatment 2,000 Units.	Results.
108	Dec. 6	Dec. 13	Dec. 13	Serum daily.	Recovered.
277	" 6	" 14	" 14	" "	Died Dec. 18
12	" 6	" 12	" 12	" "	" " 18
275	" 6	" 14	" 14	" "	" " 18
299	" 6	" 14	" 14	" "	" " 22

OBSERVATION ON SERUM SHEEP.

1. Twenty-four hours after the first injection of serum no toxin was found in the blood. This was true of all five sheep.
2. In four of the five sheep, despite the absence of toxin in the blood, the symptoms were not influenced.

CARBOLIC ACID.

Before discussing the experiments with sheep it may be of interest to give a few of the preliminary experiments with guinea-pigs. We tried first to determine how much carbolic acid was necessary to kill an average pig.

Series 1.—0.5 c.c. of a two per cent solution of carbolic acid in water was injected every two hours until 32 doses were given, without producing any effect. The amount was then increased to one cubic centimeter every two hours. Of this, 11 doses were given before the animals showed symptoms of poisoning.

Series 2.—Two other pigs weighing 275 and 256 gm. respectively were given 0.5 c.c. of a two per cent solution until 42 doses were given, without producing any effect. Treatment was then discontinued. Ten days later the pigs were alive and in good condition.

Series 3.—Five pigs were given 10×M.F.D. of U. S. government standard tetanus toxin. With the first appearance of symptoms they were given 0.5 c.c. of a two per cent carbolic solution every two hours during the day, without any apparent effect. All died.

Series 4.—Five pigs each received 10×M.F.D. tetanus toxin. The carbolic acid was given every two hours day and night, with the result that two pigs recovered and two died.

Series 5.—Carbolic injections were begun with two of the pigs immediately after the injection of the toxin with the purpose of determining whether it would prevent the development of tetanus. This it failed to do, as the pigs developed the disease and died.

Sheep Treated with Carbolic Acid.—Six sheep were used, one as control. Of the sheep treated all received two cubic centimeters of a two per cent carbolic solution every two hours day and night. All died. In comparison with the sheep in which serum alone was used, there was a very slight difference in the tonic muscular contractions, the carbolic sheep being less stiff. The difference, however, was not as marked as with magnesium sulfate and chloretone*. It had no influence on the course of the disease.

The following is a sample protocol:

Sheep 17, weight 44.5 kilos.

October 29: Inoculated in the usual way.

Eighth day: Showed stiffness in the front legs, head extended. Stump of tail extended. (This was the only sheep which showed any local tetanus.) Tail amputated 20 cm. above point of inoculation. Two cubic centimeters of a two per cent carbolic solution injected subcutaneously every two hours.

Ninth day: Symptoms more marked. Impossible to get up, but could stand when placed on feet; could not walk without falling. Could not swallow.

Tenth day: Very little change from previous day.

Eleventh day: 10 A.M. clonic convulsions. Died at 8 P.M.

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*Chlorbutanol

MAGNESIUM SULFATE.

Our results with magnesium sulfate were unsatisfactory, all of the animals dying either as a result of tetanus or as a result of magnesium sulfate poisoning. Six sheep were used, the control (No. 112) dying of tetanus. Of the five sheep treated, two were given 1.25 gm. per kilo body weight; three were given 2.50 gm. Those receiving the smaller amount showed no effect, while the latter all died apparently of magnesium sulfate poisoning.

PROTOCOLS.

Sheep 87, weight 35 kilos.

March 26. Inoculated.

Ninth day, 8:30 A.M.: Sheep slightly stiff. Tail amputated, 1.25 gm. magnesium sulfate in salt solution per kilo injected subcutaneously into abdominal wall under aseptic precautions. Fifteen minutes required for injection. During the day the sheep showed no signs of relaxation but became gradually stiffer.

Tenth day: Sheep had well marked tetanus. Unable to get up or stand when placed on feet. Magnesium sulfate repeated, but produced no effect. During the day the sheep became very stiff, legs and neck hyperextended.

Eleventh day: Dead in stall.

TABLE 6.

PIG.	Weight gm.	Inoculated with 2 c.c. on	Symptoms.	Date.	Results.
1	350	March 26	—	....	....
2	"	" 27	—	....	....
3	"	" 28	—	....	....
4	"	" 29	+	April 1	Recovered.
5	"	" 30	+	" 1	"
6	"	" 31	+	" 1	"
7	"	April 1	+	" 2	Died.
8	"	" 2	+	" 3	"
9	"	" 3	+	" 4	"
10	"	" 4	+	" 5	"
11	"	" 5	+	" 6	Recovered.

Protocol of sheep which died from magnesium sulfate poisoning:

Sheep 268, weight 32 kilos.

March 26. Inoculated.

Eighth day: Sheep showed slight signs of stiffness but moved about without difficulty. Tail amputated. 2.5 gm. per kilo of body weight of magnesium sulfate in salt solution injected subcutaneously into abdominal wall under aseptic precautions. At 12:00 the sheep was lying on its side in the stall completely anesthetized. There was relaxation of all muscles. When raised from the floor the animal was quite limp. There was a considerable amount of froth around the mouth and mucous exudate from the nose. Respirations were shallow and infrequent, 12 per minute. This condition was unchanged at 5 P.M.

Ninth day: Sheep dead in stall.

TABLE 7.

Pig.	Weight gm.	Inoculated with 2 c.c. on	Symptoms.	Date.	Results.
1	350	March 26	—	....	....
2	"	" 27	—	....	....
3	"	" 28	—	....	....
4	"	" 29	+	April 2	Recovered.
5	"	" 30	−	" 2	"
6	"	" 31	−	" 3	Died.
7	"	April 1	+	" 3	"
8	"	" 2	+	" 3	"
9	"	" 3	+	" 4	Recovered.

CHLORETONE.*

In administering chloretone* to sheep we met with many difficulties. The drug is very slightly soluble in water, 0.8 of one per cent, and somewhat less in normal salt solution. For this reason it was impossible, on account of the amount required, to give it subcutaneously or intravenously. A stomach tube can be passed with little difficulty on a sheep which does not have tetanus. In this way sufficient chloretone* can be given in a dilute alcoholic solution. However, when the animal has tetanus, the passage of a stomach tube frequently causes prolonged contraction of the muscles of respiration, leading to death. In our preliminary work several were killed in this way. Chloretone* is readily soluble in hot olive oil and remains in solution when the oil is cool. Taking advantage of this fact, we injected chloretone* dissolved in oil intraperitoneally. By this method the animals were affected almost as quickly as when the drug was given per stomach. Unfortunately, however, the repeated punctures necessary in a prolonged treatment in many cases cause peritonitis. Although conducted under aseptic precaution it was not always possible to avoid perforating the gut. In one series we treated the infected animals with chloretone* alone and in another with chloretone* in combination with serum. The series treated with chloretone* alone is as follows:

Six sheep were used, one as control, No. 301. Of these, two died of peritonitis due to the perforation by the injecting needle. One died probably of chloretone* poisoning. Two recovered.

The protocols of those dying of peritonitis are not instructive beyond the fact that the muscular symptoms of the tetanus were entirely controlled and the amount of toxin present in the blood was not affected.

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*Chlorobutanol.

The protocol of the sheep dying of chloretone* poisoning is as follows:

Sheep 46, weight 36 kilos.

July 1. Inoculated in usual way.

Seventh day: Sheep slightly stiff, particularly the neck, which he seemed disinclined to move. Tail amputated. Seven grams of chloretone* in 100 c.c. of olive oil injected intraperitoneally. One-half hour after injection the sheep was lying on its side apparently asleep. Reflexes, however, were present. Pressure in amputation stump caused slight movement.

Eighth day: In same position and apparently in same condition as day before. Unable to stand and refused to drink.

Ninth day: Sheep lying down but could be easily aroused. Drank a little water with an effort probably due to position. During the day the sheep became wider awake and showed stiffness of forelegs and neck.

5 P.M.: Intraperitoneal injection of 7 grams of chloretone* in 100 c.c. of oil.

5:30 P.M.: The animal was completely anesthetized. No eye reflex.

Tenth day: Sheep found in same position as left the night before and dead. Death probably due to chloretone* poisoning.

The guinea-pigs injected with blood from this sheep showed the usual results.

The protocols of the two sheep recovering were similar.

Sheep 37, weight 34 kilos.

Inoculated July 1.

Seventh day: Sheep quite stiff. Tail amputated. Intraperitoneal injection of four grams of chloretone* in 100 c.c. of olive oil. One hour later the sheep was lying on one side asleep but could be easily aroused. All rigidity of muscles absent. Remained this way during the day.

Eighth day: Sheep lying down but aroused easily. Drank about one-half liter of water. Refused to eat. Fur grams of chloretone* given intraperitoneally. Sheep remained sleeping during the day.

Ninth day: Sheep in same condition as previous day, not disturbed. No chloretone.*

Tenth day: Not moved from position in which he was placed previous day. Drank a small amount of water. No chloretone.*

Eleventh day: Sheep awake but unwilling to move. Refused food but drank freely about 1,500 c.c. of water. During the afternoon showed slight return to stiffness.

Twelfth day: Stiffness more marked. Drank water but refused to eat. Four grams of chloretone* injected intraperitoneally.

Thirteenth to fifteenth day: Sheep remained quietly sleeping. On the fourteenth day drank a little water. On the morning of the fifteenth day was awake and apparently hungry but could not swallow food. There was no sign of muscular rigidity. From this time the animal continued to drink and on July 17 ate well. There was no sign of the tetanus from this time.

Pigs injected are as follows:

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*Chlorbutanol.

TABLE 8.

RESULTS OF INOCULATION WITH BLOOD OF SHEEP 37.

PIG.	Weight.	Inoculated with 2 c.c. on	Symptoms.	Date.	Results.
1..................	350	July 1	—	....	....
2..................	"	" 2	—	....	....
3..................	"	" 3	—	....	....
4..................	"	" 4	+	July 7	Recovered.
5..................	"	" 5	+	" 7	"
6..................	"	" 6	+	" 8	"
7..................	"	" 7	+	" 8	Died.
8..................	"	" 8	+	" 9	"
9..................	"	" 9	+	" 10	Recovered.
10..................	"	" 10	+	" 11	"
11..................	"	" 11	+	" 12	"
12..................	"	" 12	+	" 14	"
13..................	"	" 13	+	" 15	"
14..................	"	" 14	+	" 16	"
15..................	"	" 15	+	" 17	"
16..................	"	" 16	+	" 19	"
17..................	"	" 17	—	....	....
18..................	"	" 18	—	....	....
19..................	"	" 19	—	....	....
20..................	"	" 20	—	....	....

CHLORETONE* AND SERUM.

Six sheep were used, one as control, No. 163. Of the five treated one died of peritonitis due to perforation. Four recovered.

The protocol of the sheep which died is as follows:

Sheep 300, weight 33.5 kilos.

Inoculated as usual July 13.

Seventh day: Showed slight stiffness in the forelegs. Tail amputated. Five grams of chloretone* in 100 c.c. of olive oil injected intraperitoneally and 2,000 units of serum injected intravenously. During the injection the sheep got away from the attendant and was kept on the table with difficulty. One hour after the injection the sheep was lying on its side asleep. During the afternoon it remained quiet but at 5:30 P.M. abdomen was distended.

Eighth day: Sheep remained quiet but abdomen was greatly distended. 2,000 units of serum injected.

TABLE 9.

RESULTS OF INOCULATION WITH BLOOD OF SHEEP 300.

PIG.	Weight.	Inoculated with 2 c.c on	Symptoms.	Date.	Results.
1..................	350	July 13	—	....	....
2..................	"	" 14	—	....	....
3..................	"	" 15	—	....	....
4..................	"	" 16	+	July 18	Recovered.
5..................	"	" 17	+	" 19	"
6..................	"	" 18	+	" 19	Died.
7..................	"	" 19	+	" 20	"
8..................	"	" 20	+	" 22	Recovered.
9..................	"	" 21	—	....	....

*Chlorbutanol.

Ninth day: Sheep dead in stall.

Autopsy showed three perforations of the gut, and in the exudate a streptococcus and a long bacillus were found.

TABLE 10.

Results of Inoculation with Blood of Sheep 110.

Pig.	Weight.	Inoculated with 2 c.c. on	Symptoms.	Date.	Results.
1	350	July 13	—	....	....
2	"	" 14	—	....	....
3	"	" 15	—	....	....
4	"	" 16	+	July 18	Recovered.
5	"	" 17	+	" 19	"
6	"	" 18	+	" 19	Died.
7	"	" 19	+	" 20	"
8	"	" 20	+	" 21	"
9	"	" 21	—	....	Recovered.
10	"	" 22	—	....	"
11	"	" 23	—	....	"
12	"	" 24	—	....	"
13	"	" 25	—	....	"
14	"	" 26	—	....	"
15	"	" 27	—	....	"
16	"	" 28	—	....	"
17	"	" 29	—	....	"
18	"	" 30	—	....	"
19	"	Aug. 1	—	....	"
20	"	" 5	—	....	"

The following is a protocol of one of the four sheep which recovered:

Sheep 110, weight 36 kilos.

Inoculated as usual on July 13.

Seventh day: Sheep slightly stiff in forelegs and neck. Tail amputated. Five grams of chloretone* injected intraperitoneally and 2,000 units of serum intravenously. One hour later sheep was lying quietly on its side asleep, relaxation complete.

Eighth day: In the same position as previous day, completely relaxed. 2,000 units of serum injected. During the day sheep remained quiet, but at 5:30 p.m. began to wake up but refused to drink.

Ninth day: Sheep well awake and slightly stiff. Drank about one liter of water but refused food. Five grams of chloretone* in 100 c.c. of olive oil injected intraperitoneally and 2,000 units of serum intravenously. Went to sleep immediately and remained so during the entire day.

Tenth day: In same position as previous day and asleep. No stiffness. 2,000 units of serum injected intravenously.

Eleventh day: Remained lying on side during the day but could be aroused, and at 5:30 p.m. drank a little water. 2,000 units of serum. No chloretone.*

Twelfth day: Sheep awake and slightly stiff. 2,000 units of serum injected intravenously. During the day the sheep tried to get up but was unable to because of stiffness which had increased to a considerable extent.

5 p.m.: Intraperitoneal injection of 5 grams of chloretone.*

Thirteenth to fifteenth day: Sheep remained sleeping quietly. At 5:30 p.m., July 27, could be aroused and drank about 1,500 c.c. of water. On each of these days 2,000 units of serum were given intravenously.

Sixteenth day: Sheep awake but not stiff. During the day remained drowsy but the stiffness did not return as on previous days.

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*Chlorbutanol.

Seventeenth day: Sheep awake, could stand when placed on feet but was very weak. Drank well and ate a small amount.

Eighteenth day: Improved in all conditions. No stiffness and walked a little. Sheep rapidly recovered strength and on August 5 was in normal condition.

The pigs injected were as given in table 10.

CONCLUSIONS.

It appears to us that the following conclusions are warranted by these experiments:

1. Amputation after the appearance of symptoms is of no value.

2. The toxin appearing in the blood stream is self-limited even in the fatal cases.

3. There is little if any value in the carbolic acid treatment of the disease.

If there is any gain, our opinion is that it is probably due to the sedative action of the drug and not to any direct action on the disease process, and that this result may be obtained with greater certainty by other drugs.

4. The magnesium sulfate as used in our experiments, subcutaneously, is of no value.

5. Antitetanic serum alone has a definite, although usually insufficient, curative effect.

6. It appears to us, from the observation of a large number of animals and quite a number of human beings dying of tetanus, that the exhaustion due to the muscular contractions is a large factor in producing fatal results. For this reason much of our work has been given to the attempt to hold these convulsions in check.

7. The presence of a large amount of toxin in the blood several days (in sheep it can be demonstrated four days) before the onset of clinical symptoms makes it imperative that a method be devised for easily determining this. With such a method it is quite probable that we could save a large proportion of our tetanus cases.

8. With our present knowledge of the subject, the best that can be done in the treatment of tetanus is to neutralize the toxin with repeated doses of serum while controlling the muscular spasm with some such drug as chloretone.*

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*Chlorbutanol.

SPIROCHAETA SUIS, ITS SIGNIFICANCE AS A PATHOGENIC ORGANISM.*

STUDIES ON HOG CHOLERA.

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(WITH PLATE 4.)

INTRODUCTION.

Since results of the dark field study of the blood of animals suffering from hog cholera were reported,[1] the work has been continued and several new phases of the problem have been investigated. In the former article it was reported that a relatively large spirochete and characteristic granules were present in the blood of 40 hogs infected with cholera, representing 12 different strains of virus, while the blood of 48 normal hogs were observed to be free from spirochetes and granules. The spirochetes observed in specimens of blood from cholera hogs were very few in number, but the characteristic granules were apparently always present during the fastigium and were regarded as important findings.

In conjunction with the later studies of this spirochete in the blood, many observations were made by means of the dark field of material taken from the intestinal ulcers of hogs dead from hog cholera. Dark field examinations have been made of the necrotic tissue from the intestinal ulcers of all animals dead from hog cholera during the course of our experiments of the last six months. Scrapings from the intestinal mucosa and contents of the crypts in the ceca of normal hogs, some of them being susceptible to hog cholera, others immune to the disease, were also examined at every opportunity. In all, the study with the dark field of material from the crypts and ulcers of normal and diseased hogs has included about 140 cases. As a result of this work, the details of which will be published separately, we are inclined to believe that the following observations are true:

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[1] King, Baeslack, and Hoffmann, *Jour. Infect. Dis.*, 1913, 12, p. 206.

1. The mucosa of the large intestine, particularly that of the cecum, of normal hogs, includes in its flora non-pathogenic spirilla and spirochetes, relatively large forms predominating.

2. In the ulcerated patches of cecal mucosa and in the crypts, near the ileo-cecal valve, of hogs dead from cholera is localized a constant species of spirochete, which is pathogenic for swine and which plays an important part in the production of hog cholera.

3. The crypts and healed ulcers of hogs actively immunized against hog cholera may contain, for a variable period of time after immunization, in addition to non-pathogenic spirochetes, the same species of pathogenic spirochete referred to above.

There are many factors entering into the above tentative conclusions which cannot be discussed in detail in this paper, but which will follow in future reports of our studies on this disease. Among the more important observations, however, are the results obtained from the experimental curative treatment of cases of hog cholera with arsenical and mercurial preparations. This work results, not in cures, but, usually, in prolongation of life, which, in most cases, was coincident with an apparent decrease in number or total disappearance of the spirochetes in question from the necrotic tissue in the cecal ulcers. Positive dark field findings of spirochetes in intestinal ulcers of cholera hogs are in accordance with the results reported by von Rüther,[1] Uhlenhuth and Haendel,[2] and Betegh.[3] The presence of spirochetes in the bile of cholera hogs, as reported by Uhlenhuth and Haendel, has not been found in the cases examined thus far.

On account of the fact that efforts to secure cultures or satisfactory stained preparations of *Spirochæta suis* from the blood of cholera hogs were not successful, and because of the large amount of bacterial contamination present in the intestinal lesions, attempts were made to find localized foci of the organism elsewhere in the diseased body. Microscopical preparations and cultures were made from the spleen, liver, lymphatic glands, spinal cord, cerebrospinal fluid, bile, and urine. These attempts were unsuccessful except in one instance. In one case (Hog 615, inoculated subcutaneously with exudate from local lesion, Hog

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[1] [illegible] 1911, [illegible] p. [illegible]

[2] [illegible] 1913, 6, p. [illegible]

[3] [illegible] 1912, [illegible] p. [illegible]

599), the dark field revealed in the inguinal lymph gland a spirochete resembling the assumed pathogenic form. In many of the dark field preparations numerous granules were observed, but it was impossible to draw any definite conclusions as to their origin and relationship to *Spirochæta suis* or to tissue elements.

Finally, when an opportunity presented itself, dark field examinations were made of external lesions on the leg and ear of a case of cholera of the chronic type (Hog 583, May 8, 1913) and they were found to contain spirochetes, presumably of one species, in large numbers. So far as we know, similar observations have never been reported except by Dodd[1] of Pretoria, Transvaal. In describing "a disease of the pig due to a spirochete," Dodd states that he found a spirochete in the cutaneous lesions of a number of pigs. The disease could not be transmitted experimentally by the inoculation of blood from an infected animal, but was transmitted, however, by actual contact. The infection was fatal to several pigs. Autopsy revealed nothing except the local skin lesions and general anemia. The disease appeared to be some cutaneous infection due to the spirochete found in the local lesions. Scrapings from the skin of healthy hogs and from the normal skin of the affected pigs were found to contain no spirochetes.

Since the first observation we have not only found that all external local lesions examined, including 11 cases of hog cholera, were constant foci for *Spirochæta suis,* but we have also been able to produce typical hog cholera, with local external lesions, by inoculating a small bit of the tissue or a small amount of exudate from local lesions of the affected animal into a healthy pig. Scrapings from the healthy skin of hogs and from the areas of discoloration on the ventral surface of the body of animals suffering from cholera have been submitted to dark field examination with negative results. The results of this work, which is still in progress, will be published later.

In our former paper, the spirochete observed in the blood of hogs infected with hog cholera was described as follows:

> "In the specimens of blood from all infected hogs, which have been observed by means of the dark field, a relatively large spirochete has been found. It averages from five to seven microns in length and one

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[1] *Jour. Comp. Path. and Ther.,* 1906, 19, p. 216.

micron in width. The body of the organism is flexible and round at its ends. It presents no knobbed appearance at its poles. Actively motile, it revolves about its longitudinal axis. Its motility is undulating in character and its spirals are fixed. A few of these organisms have been observed dividing longitudinally. In one permanent microscopical mount, prepared by india-ink fixation, one of these organisms apparently shows a polar flagellum. On the dark field this spirochete is readily distinguished from bacteria on account of its lack of rigidity and its characteristic motility, and from 'blood filaments' by its greater refractive properties and characteristic morphology.

"This spirochete has not been found in large numbers in any of the blood preparations. However, in nearly every specimen examined more than one has been observed and in many cases five or six have been found with little difficulty. As a rule the organisms have been found to be more numerous at the height of the disease. The specimens of blood examined have been diluted in the proportion of about one to ten or fifteen with sterile sodium citrate solution, which factor should be considered in contemplating the number observed in a given positive specimen. Moreover, it is suggested that this organism, when observed as a spirochete form, constitutes only one stage of its development."

This description appears to be correct, except that we have never verified the presence of a polar flagellum.

It must be conceded that any successful dark field investigation requires patient work and much practice in interpretation. Therefore, one unacquainted with the dark field should not expect to obtain immediate satisfactory results from the study of blood. An intimate course of study is necessary to enable one to differentiate between "filaments," artefacts, and organisms of the morphological nature of the spirochete described in the blood of cholera hogs.[1]

The last statement quoted we now accept as a conclusion rather than a suggestion. Results lead us to believe that the spirochete observed in few numbers in the blood of cholera hogs is a transitional form.

The spirochete, which is constantly observed in the ulcerated mucosa and crypts in the ceca and in the external local lesions of cholera infected animals, is an active motile, comparatively long, spiral-shaped organism, of slight thickness. Measurements show that it is approximately 6-10 microns in length, averaging about 7 or 8 microns, and 0.3-0.6 micron in width. The convolutions vary from three to ten in number, rarely more than eight, and the spirals are fixed. It has been observed to divide longitudinally, and morphological variations, such as described by Gleitsmann[1] as a part of the life cycle of the Sudanspirochætæ, have been observed.

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[1] In this connection we were especially fortunate in the collaboration of our colleague, Dr. F. W. Baeslack. Because of his experience with the dark field in his study of *Spirochæta pallida* (see *Jour. Infect. Dis.*, 1913, 12, p. 55) and in his experimental observations of certain Trypanosomes, our dark field study of hog cholera blood was placed to great advantage. It is a pleasure to acknowledge his valuable assistance in that work, as well as the many helpful suggestions he has offered in our present investigation.

In our former report on the dark field study of the blood, observations relative to the presence of granules were summarized as follows:

> "Certain types of granules appear to be characteristic of blood from cholera hogs. It usually contains many granules, some very fine yet more distinct than blood dust, some larger still, and some very distinct, highly refractive bodies. In many specimens of cholera blood were observed innumerable small granules, which were much more definite and distinct in outline than blood dust, and easily differentiated from the whitish, partially refractive granules from ruptured leukocytes and from the more highly refractive and larger bodies composed of débris, bacteria, and filaments. In this work the presence of these granules appeared to be so characteristic of blood from cholera hogs that it became an invaluable aid in finding the spirochetes. While these particular bodies may be disintegrated blood elements resulting from disease processes, yet it may properly be suggested that some of them may represent certain stages in the life cycle of the spirochete which has been observed."

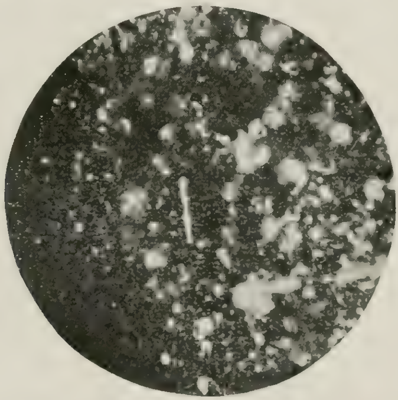

FIG. 1.—Photomicrograph of *Spirochæta suis* in exudate, ear lesion, Hog 626. ×1200. (Dark field preparation.)

As the result of continued investigations, we believe that the granules represent an important factor in the life history of *Spirochæta suis*. References to similar observations in the study of various spirochetes are found in the writings of Balfour,[2] Möhlens,[3] Ross,[4] McDonagh,[5] Dutton,[6] Mayer,[7] Mackinnon,[8] Schellack,[9] Bosanquet,[10] Marchoux and Couvy,[11] and others.

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[1] *Centralbl. f.* I, *Orig.*, 1913, 68, p. 31.

[2] *Report IV, Wellcome Research Lab.*, p. 76.

In cultures made from material containing *Spirochæta suis* the presence of small granular forms is quite constant. As these appear in dark field preparations from cultures, they resemble those which were observed in the blood, and are seen in the exudate from local lesions, of cholera-infected hogs. They are relatively highly refractive, smaller than cocci, peculiarly distinct in appearance from foreign material, and possess more than the characteristic Brownian movement. The assumption that these granules form an integral part of the biologic processes of the organism, *Spirochæta suis*, is substantiated by the results of the detailed experiments which follow.

Recognizing the difficulties involved in attempting to study *Spirochæta suis*, and in determining its possible significance as a pathogenic form, our efforts have been directed toward the cultivation of the organism. This work has included the inoculation of artificial culture media with blood, intestinal ulcers, urine, contents of local external lesions, and other tissues from cases of hog cholera, followed by careful dark field examination.

CULTIVATION OF SPIROCHÆTA SUIS.[12]

A great many plantings on a variety of artificial culture media have been made from the blood, intestinal ulcers, and external local lesions of cholera-infected animals. These cultures have been made chiefly in deep horse serum media, to which have been added various chemicals in different proportions. All have been grown under strict anaerobic conditions in special electric incubators. Various means were used in attempting to eliminate contaminating organisms, which were always present in cultures made from the ulcers and, to a lesser extent, in those made from material in local lesions.

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[3] *Handbuch d. Path. Protozoen, Prowazek*, Leipzig, 1912, p. 361.

[4] *Brit. Med. Jour.*, 1912, p. 1651.

[5] *Lancet, Proc. Roy. Soc.*, 1913, 6, p. 86.

[6] *Jour. Trop. Med.*, 1907, 10, p. 385.

[7] *Arch. f. Schiffs.-u. Tropenhyg.*, 1908, 12, Beihefte I, p. 1.

[8] *Parasitology*, 1909, 2, p. 267.

[9] *Arb. a. d. k. Gsndhtsamte*, 1909, 30, p. 351.

[10] *Spirochætes*, Saunders, 1911.

[11] *Ann. de l'Inst. Past.*, 1913, 27, p. 450.

[12] As the proof of this paper is being read we note an article in current literature by Pfeiler and Lentz (*Berl. tierärztl. Wchnschr.*, 1913, 29, p. 690), who present the case histories of 15 pigs which died after having received injections of cultures made from the Berkefeld filtrates of serum and tissue exudates from hog cholera infected animals. These cultures represented transfers in the first, second, third, and fourth generations. The authors carefully avoid any statements which might indicate the nature of the cultures. No mention is made of the character of the organism in their cultures, the composition of their culture medium, or how long or under what conditions the cultures were incubated. On account of the conspicuous lack of important data one can form no conclusion as to their mode of procedure, and, hence, the results which they have published must be regarded with some skepticism.

The culture medium which proved most successful consisted of a mixture of horse serum and pyrogallic acid, in which was placed fresh rabbit kidney at the time of inoculation. The pyrogallic medium of Proca, Danila, and Stroe[1] was made up in lots of one liter each by adding to 900 c.c. of horse serum 100 c.c. of pyrogallic acid mixture. The pyrogallic solution should be somewhat aged. A stock of solution was therefore kept, which consisted of pyrogallic acid, 10 gm.; sodium hydroxid, 20 gm., and water, 1,000 c.c. After the addition of this mixture to horse serum and distribution in tubes the medium was heated one hour at 60° C. on each of three consecutive days.

While this pyrogallic serum medium does not eliminate all bacterial contamination, at the same time allowing the spirochete to grow, it will be found to give better results than media containing formol, gentian, violet, phenol, or other inhibitory substances. For uncontaminated material, such as Berkefeld filtrates of impure cultures of spirochetes, plain or ascitic horse serum media were utilized. Fresh rabbit kidney was added to all media used and care was exercised that the horse serum was only partially coagulated and soft in consistency when ready for use.

The cultures have been examined at different intervals of time, following inoculation and incubation, in order to detect any facts which might come to light concerning the life history, morphology, and physiological habits of the organism. The method of examining cultures consists simply in withdrawing, with a sterile capillary pipette, a small portion of culture from the lower layers of the medium and examining the same in microscopical mount by means of the dark field. The Giemsa method of staining is employed whenever permanent mounts are desired. After some practice one can readily detect, by the dark field, spirochetes and granules in fresh cultures in distinction from cocci, other bacterial contamination, crystals, and small particles of culture medium.

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[1] *Compt. rend. Soc. de biol.*, 1912, 72, p. 895.

NOTES ON TRANSMISSION OF HOG CHOLERA BY MEANS OF CULTURES OF SPIROCHÆTA SUIS.

I. ORIGINAL CULTURE.

Culture 551-A was secured from the ulcerated cecum of Hog 551. The account of this case may be found in the chart for Hog 551.[1]

Cultures were made from the ulcerated cecum, spleen, and lymphatic glands of Hog 551. Those from the spleen and lymphatic glands developed no growth, while some of those from the cecum, after five days' incubation under anaerobic conditions, showed the growth of spirochetes which varied in form and size. Fig. 3, p. 212, in our former publication[2] shows a photomicrograph of a stained preparation made from this culture. When this culture was studied, our attention was directed especially to the larger forms of spirochetes which were found in it. This was due to the fact that we were familiar with the form which had been observed in the blood of cases of hog cholera. At this time no special attention was given to smaller forms representing more typical spirochetes which were present in this culture, as was shown, some weeks after the study of the culture, by careful re-examinations of the permanent stained preparations.

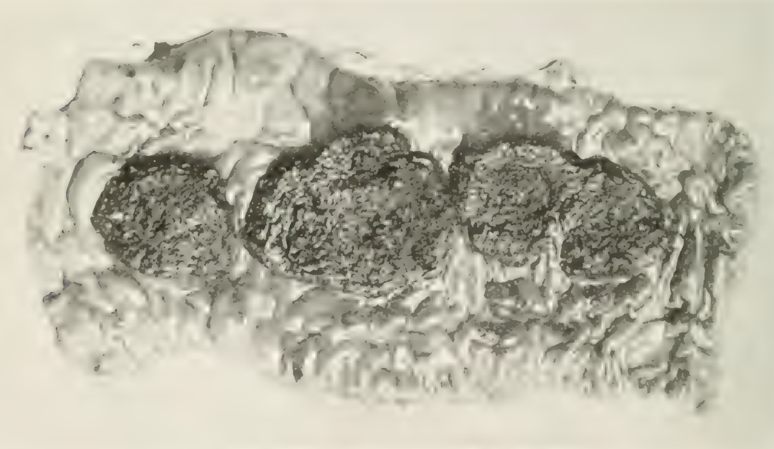

Fig. 2.—Ulcers in cecum of Hog 569.

Transfers were made from Culture 551-A and grown both aerobically and anaerobically. The species of bacteria which contaminated Culture 551-A were very carefully isolated and identified as follows: *B. pyocyaneus,* three strains; *B. coli communis,* five strains. These cultures, which contaminated 551-A, representing *B. coli communis* and *B. pyocyaneus,* were mixed and suspended in physiologic salt solution. Six cubic centimeters of this suspension on March 14 were injected intravenously into Hog 570. This animal showed no disposition to develop hog cholera, but did react to the injection of live *B. pyocyaneus* and *B. coli communis,* as the clinical chart for Hog 570 will show.

Culture 551-A was suspended in sterile physiologic salt solution and an intravenous injection of 10 c.c. given to Hog 559. The results of this inoculation are recorded in the chart for Hog 559.

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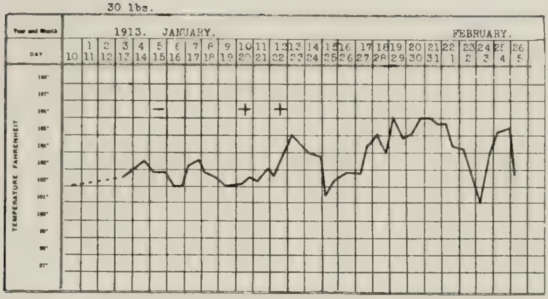
[1] Positive and negative signs indicate the presence or absence of *Spirochæta suis* in the blood of the animal on dates indicated.

[2] Jour. Infect. Dis., 1913, 12, p. 206.

Hog 551.



January 10. Received from Rochester, Mich.

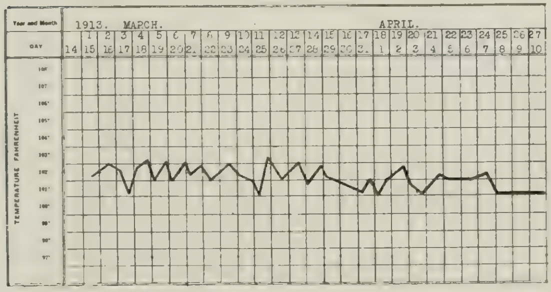
January 16. Intramuscular injection 8 c.c. of virus, Berkefeld filtered, 1:4 saline. Ohio (Pettigrew) strain.

January 31. Animal very weak.

February 5. Animal moribund, bled and examined.

Typical lesions of cholera: lungs, large areas of hepatization; kidney, petechiated, capsule non-adherent; lymphatic and inguinal glands, greatly enlarged and hemorrhagic; spleen, friable and engorged with blood, normal in size; cecum, large area of ulcers.

Hog 570.



March 14. Intravenous injection of 6 c.c. physiologic salt suspension cultures from cecum of Hog 559.

March 18-25. Slightly indisposed, appetite not good, otherwise condition practically normal.

March 31. Apparently in good condition.

April 2. A little "off feed."

April 9. Fully recovered, released and put on other work April 14.

April 30. Animal found dead, result of intramuscular injection of 5 c.c. II Cycle virus. Typical lesions of hog cholera.

Hog 559 was in a perfectly healthy condition at the time of inoculation, February 10, and weighed approximately 75 pounds. Four days after inoculation the temperature of the animal began to rise and on the fifth day symptoms of hog cholera appeared. The animal was slightly "off feed" and inclined to be inactive. Dark field examination of the blood at this time showed the presence of a few spirochetes and characteristic granules. After 48 hours the temperature dropped somewhat and for several days ranged between 103° and 104°. About 12 days after the inoculation a remission occurred, the temperature was elevated 2° during one afternoon, and from this time the animal gradually grew worse until death, which occurred 22 days after inoculation. The later symptoms consisted of weakness, inability to use hind quarters, bloody urine, mucous diarrhea, anorexia, and general malaise. On March 4 the animal was moribund, and after being photographed was bled from the carotid artery and examined (see photograph of Hog 559). On autopsy typical lesions of hog cholera were found.

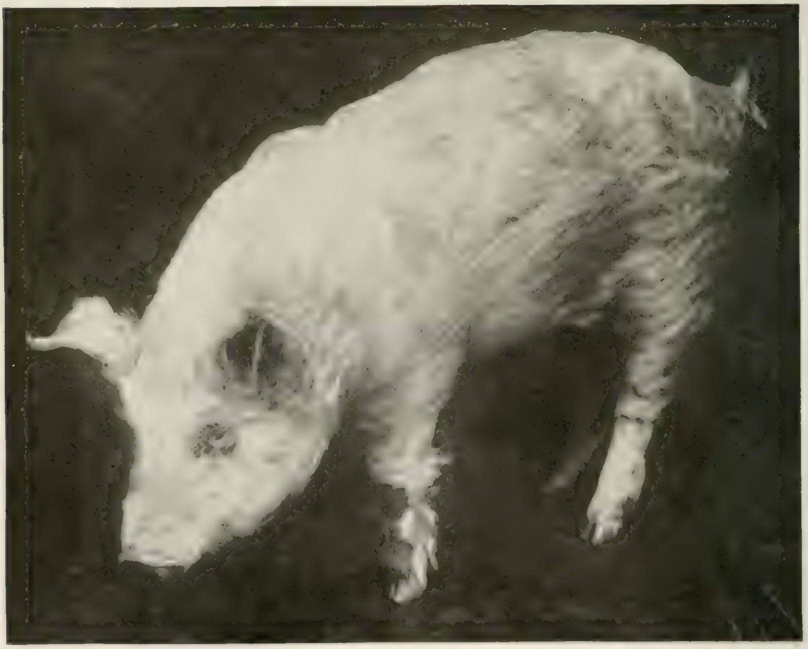


Fig. 3.—Hog 559.

Blood extravasations were present in the subcutaneous tissue, especially in the axillary region and lower abdominal region. Both the auricles and ventricles of the heart showed ecchymosis. The lungs contained numerous hemorrhagic infarcts and a few areas of hepatization. The liver appeared to be somewhat enlarged and contained areas of fatty de-

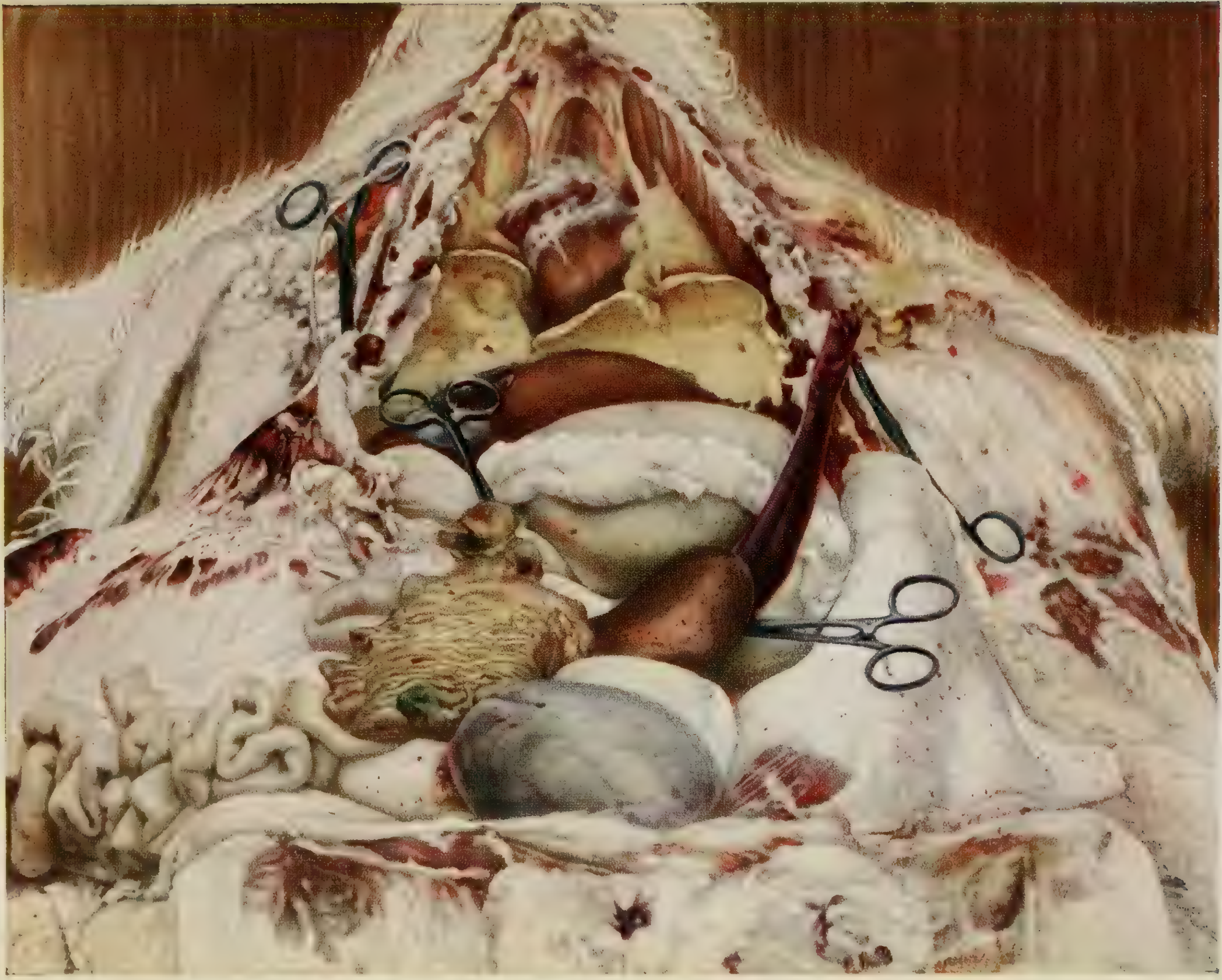
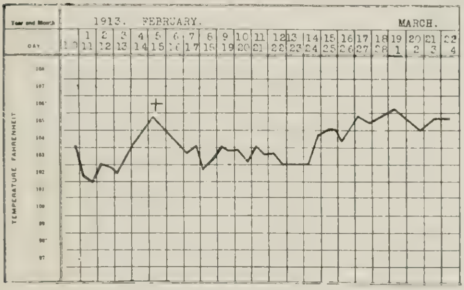


PLATE 4. HOG 559.—Inoculated 22 days previously with suspension of culture from cecum of Hog 551.

generation. The spleen was slightly atrophied, tissue firm, but engorged with blood. Both kidneys were full of petechiæ. The mucosa of the cecum and the large intestine was congested and marked with hemorrhagic spots throughout. Two typical button ulcers were found in the cecum. In the small intestine there were present two enlarged areas, consisting of necrotic intestinal wall surrounded by extensive hemorrhages. The peritoneum in the lower abdominal region showed hemorrhagic areas. The mesenteric, inguinal, and intraperitoneal lymph glands were enlarged and hemorrhagic (see Plate 4).

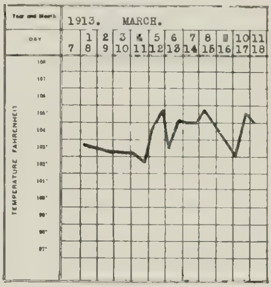
A specimen of blood serum from Hog 559 was filtered through Berkefeld, after being diluted 1:4 with physiologic salt solution, and 10 c.c. of the filtrate injected intramuscularly into Hog 569. This material proved to be virulent, as Hog 569 showed severe symptoms of hog cholera five days after inoculation and became moribund on the eleventh day. The autopsy showed classical lesions of hog cholera (see chart for Hog 569).

Hog 559.



February 10.	Intravenous injection 10 c.c. suspension physiological salt solution of Culture 551-A.
February 15.	Clinical symptoms present, appetite poor.
February 23.	Animal improved in condition.
February 25.	Remission.
March 4.	Animal moribund, and bled from carotid artery. Typical lesions: heart, lungs, kidney, cecum, and lymphatic glands.

Hog 569.



March 7.	Blood negative, free from granules.
March 7.	Intramuscular injection of 10 c.c. Virus 559, diluted 1:4, physiological salt and Berkefeld-filtered strain (Culture 551-A).
March 12.	Symptoms appeared.
March 18.	Animal moribund and bled from carotid artery. Lesions typical: heart, lungs, kidney, inguinal glands, and mesenteric lympth glands; ulcers in cecum.

With reference to infection from other material than that which was used in the inoculations, it should be stated that results from Hog 559, as well as all others used in this series of experiments, were carefully controlled by the presence of normal hogs in the experimental stable. The animals used in each series of experiments, as well as the normal animals, were kept in separate rooms. The normal animals were placed under exactly the same conditions and had the same care that was exercised in regard to the treated animals.

An additional control on this work depended upon the use of Hog 570, which was inoculated with the contamination present in Culture 551-A. Furthermore, it has been observed that the serum of Hog 559, filtered through Berkefeld, was capable of producing typical hog cholera in Hog 569.

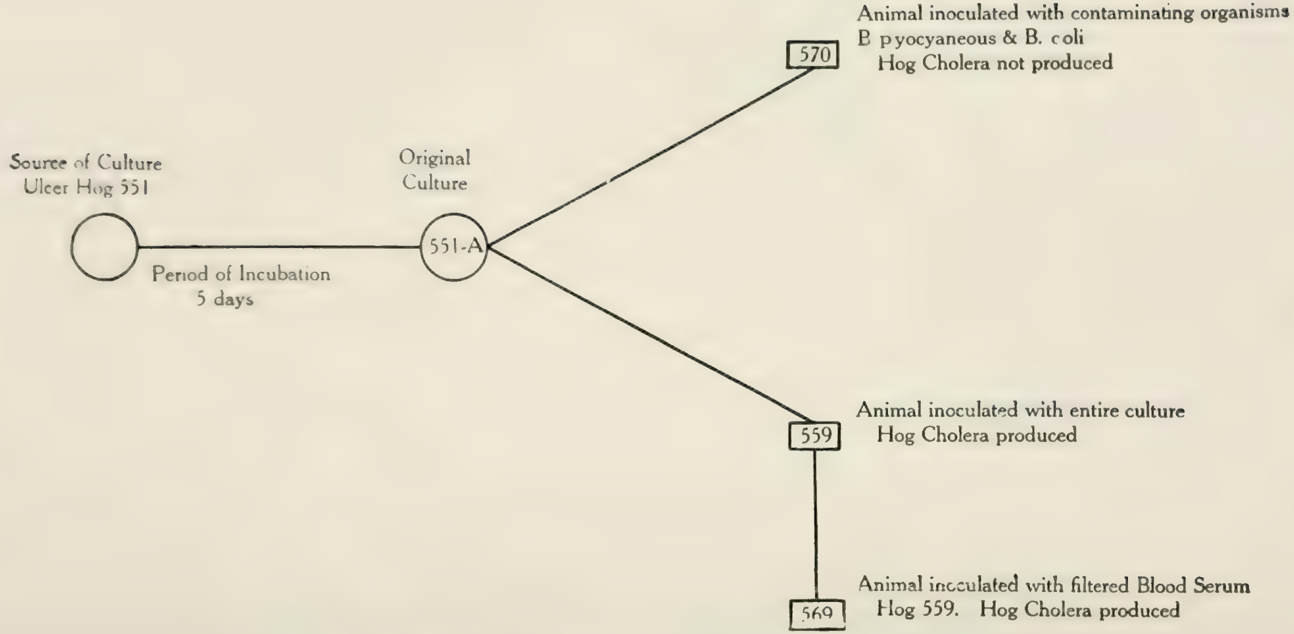

Source of Culture
Ulcer Hog 551
Period of Incubation
5 days
Original
Culture
551-A
570
Animal inoculated with contaminating organisms
B pyocyaneous & B. coli
Hog Cholera not produced
559
Animal inoculated with entire culture
Hog Cholera produced
569
Animal inoculated with filtered Blood Serum
Hog 559. Hog Cholera produced

Chart 1.—Graphic representation of experiments with original culture from Hog 551.

Among the criticisms which may be offered on this experiment, the most important relates to the possibility of transferring the original virus from Hog 551 through Culture 551-A. This point could not be controlled, although an estimate was made that Hog 559 received less than 1/750 c.c. of material originally obtained from the ulcer of Hog 551. It has been estimated by King and Wilson[1] that the minimum fatal dose of hog cholera virus, diluted in physiologic salt solution, is approximately 1/300 c.c. The above approximation that Hog 559 received about 1/750 c.c. was obtained on the basis of the utilization of about 1/15 gm. of ulcer from Hog 551 in approximately 10 c.c of culture media. However, these estimates, including both the suggested minimum fatal dose of hog cholera virus in physiologic salt solution and the amount of original material transferred through Culture 55-A to Hog 559, are open to serious question in attempts to estimate whether or not any of the original virus from Hog 551 was transferred to Hog 559.

II. TRANSFER I. SECOND GENERATION ON ARTIFICIAL MEDIA.

This culture was secured from the strain of virus from Hog 577. This animal received an intramuscular injection of 8 c.c. horse serum virus, diluted 1:4 in physiologic salt solution and Berkefeld filtered. The animal developed typical symptoms of hog cholera, and after nine days, being moribund, was bled from the carotid artery and examined.

Hog 587 on April 11 received an intramuscular injection of 5 c.c. of serum from Hog 577. This animal promptly developed hog cholera of the acute type after five days' incubation and was bled and examined eight days after inoculation. The autopsy showed typical lesions of hog cholera of the acute type. Numerous blood extravasations were present in the subcutaneous tissue, especially in the axillary region. Both lungs were almost completely consolidated. The kidneys showed areas of cloudy swelling and petechiæ near the upper ends. The spleen was engorged with blood. The mesenteric and inguinal glands were enlarged and hemorrhagic. The cecum contained a few small ulcers around the valve (see chart for Hog 587).

On April 19 cultures were made from the material obtained from the small ulcers in the cecum of Hog 587. These were incubated under anaerobic conditions for a period of 17 days and on May 5 dark field examination showed the presence of *Spirochæta suis*. A variety of contaminating organisms was also present, both cocci and bacilli being represented in the contamination. The culture medium in the tube labeled "587-A" showed no liquefaction from the growth of the contaminating bacteria, and from it a number of transfers were made on May 5. On May 27, after incubation for 22 days, transfer labeled "Transfer 1-587-A," when submitted to dark field examination, showed a few *Spirochætæ suis* and numerous granules. All of the contaminating bacilli which were

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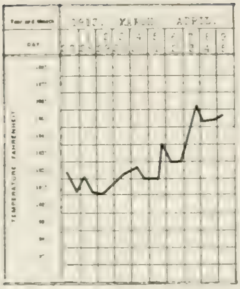
[1] *Bull. 171, Kansas State Agric. Coll.*, p. 163.

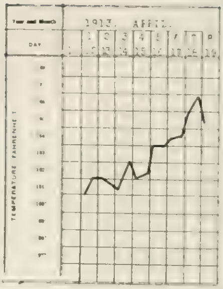
Hog 577.



March 27. Intramuscular injection 8 c.c. activated horse serum, diluted 1:4, physiological salt solution and Berkefeld filtered.
April 1. Symptoms appeared.
April 3. Animal very weak in hind quarters.
April 5. Animal moribund, bled and examined.

Typical lesions: heart, lungs, kidney, lymph glands, cecum; small intestine infected with *Ascarida suilla*.

Hog 587.

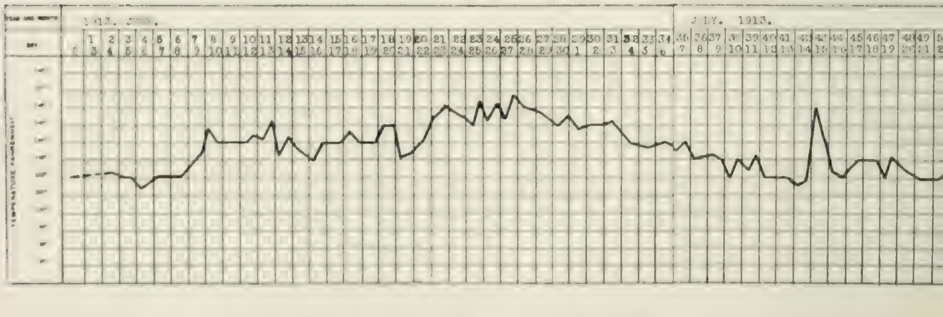


April 11. Intramuscular injection of 5 c.c. of Virus 577 I Cycle activated horse serum.
April 16. Symptoms of cholera appeared.
April 19. Animal moribund, bled from carotid artery and examined.

Lesions: lungs, spleen, cecum, subcutaneous tissue, and inguinal glands.

present in the original culture, 587-A, had disappeared and the only contamination present in the transfer was a micrococcus. A portion of this culture, Transfer 1-A-587-A, was broken up in sterile water and 1.5 c.c. of the suspension of this culture was inoculated intramuscularly into each of three hogs, Nos. 612, 613, and 614. Hogs 612 and 614, after eight days' incubation, developed symptoms of hog cholera. After a few days the disease assumed the chronic form. The animals lost their normal appetite, and during a period of about three weeks following the initial

Hog 612.



June 2. Intramuscular injection 1.5 c.c. suspension Culture Transfer 1-587.
June 10. Symptoms appeared.
June 10-July 1. Chronic case of cholera.
July 4. Condition improved.
July 15. Animal had been fighting with strange hogs.
July 22. Released as healthy immune.

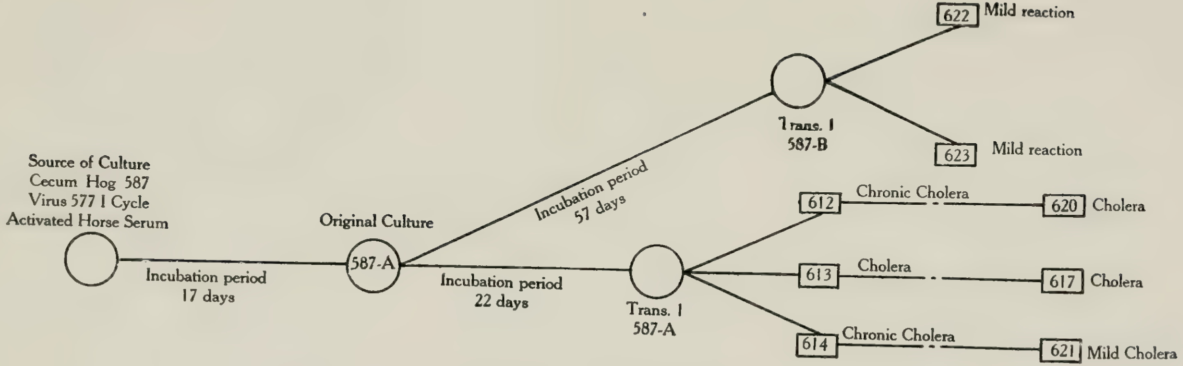
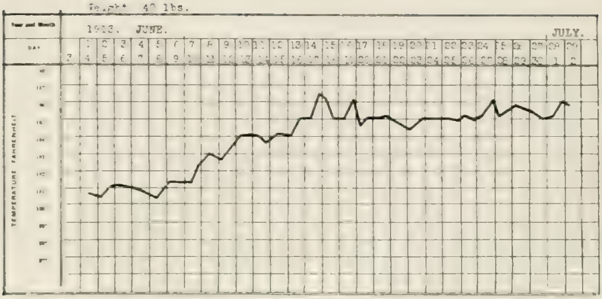


CHART 2.—Graphic representation of experiments with Transfer 1-587.

Hog 613.

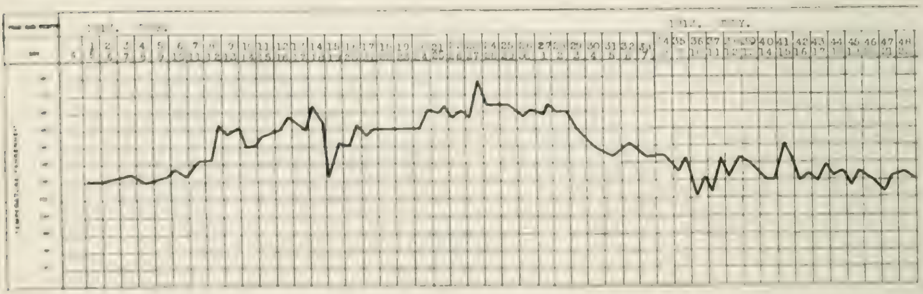


June 3. Intramuscular injection of 1.5 c.c. suspension Culture Transfer 1-587.
June 10. Symptoms appeared.
June 16-July 2. Subacute type of cholera.
July 2. Animal moribund, killed and examined.
Typical lesions.

symptoms became somewhat emaciated. On the fifth week after the inoculation of the animals rapid convalescence took place and they fully regained normal condition. Both animals had been confined with cholera hogs, but following the experiment they remained healthy and were released as active immunes.

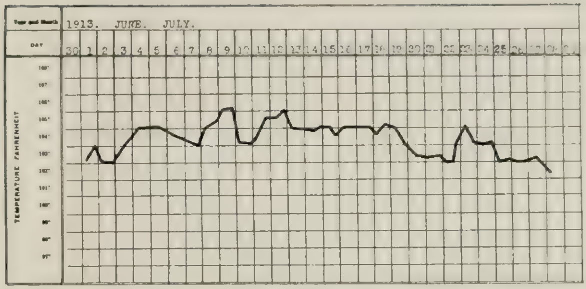
Hog 613 developed a more acute form of the disease than Hogs 612 and 614. After an incubation period of eight days, the temperature daily rose and maintained an elevation of approximately 106° to 107° for a period of two weeks. During this time Hog 613 manifested the usual symptoms of hog cholera, consisting of anorexia, general weakness and malaise, paralysis of hind quarters, emaciation, and constipation. On July 2, 29 days after inoculation, the animal was killed; on examination,

Hog 614.



June 3. Intramuscular injection 1.5 c.c. suspension Culture Transfer 1-578.
June 12. Animal "off feed."
June 15-30. Symptoms of chronic type of cholera.
July 4. General improvement noted.
July 15. Animal had been fighting, placed with strange hogs.
July 22. Released as healthy immune.

Hog 620.

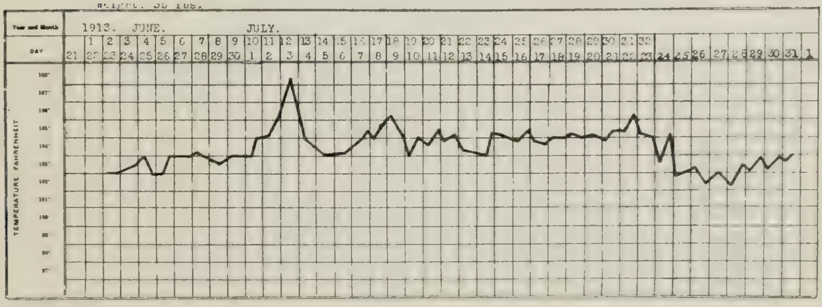


June 30. Intramuscular injection of 10 c.c. of blood of Hog 612, diluted 1:4 and Berkefeld filtered.
July 4-5. High temperature—may have been due to excessively hot weather.
July 8. Symptoms of cholera.
July 10-20. Gradual emaciation, constipation, anorexia, and malaise.
July 21. Gradual return of appetite and slow convalescence.
July 29. Apparently recovered.
July 30. Animal released as normal.

characteristic lesions of hog cholera were found. The heart was normal, the lungs were congested and contained areas of hepatization. The spleen was enlarged, soft, and friable, and engorged with blood. The kidneys were petechiated. The mesenteric and lymphatic glands were enlarged and hemorrhagic, and the congested mucosa of the cecum contained small ulcerated patches near the ileocecal valve. *Spirochæta suis* was present in the ulcers.

This experiment was controlled in the manner explained in the description of the inoculation of Hog 559 with original culture. Normal hogs were kept in the stable under the same conditions as were these animals. Hogs 612 and 614, which survived the experiment, possessed active

Hog 617.



June 21. Intramuscular injection of 12 c.c. of blood of Hog 613, diluted 1:4, and Berkefeld filtered.
July 21. Gradual return of appetite and slow convalescence.
June 27. Symptoms appeared.
July 1-15. Animal very weak, emaciated, constipated, anorexia, and malaise.
July 20. Some improvement noted.

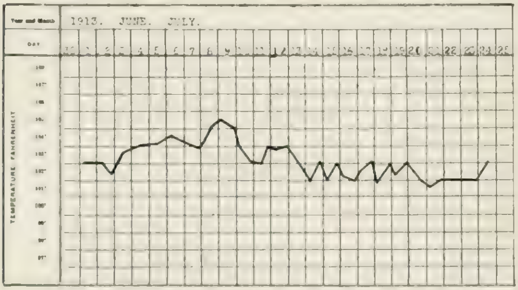
immunity, as was demonstrated by the fact that they were placed with hogs sick with cholera two weeks before being released.

During the period of infection a sample of blood was obtained from the tail of each of Hogs 612, 613, and 614, used in the experiment above in testing the pathogenicity of Transfer 1-587-A. These samples were defibrinated, diluted 1:4 in sterile water, and passed through the Berkefeld filter. Ten cubic centimeters of the diluted, Berkefeld-filtered blood from Hogs 612, 613, and 614 were injected intramuscularly into Hogs 620, 617, and 621, respectively.

Hog 620, which received the Berkefeld-filtered blood serum from Hog 612, developed symptoms of cholera eight days after inoculation. This case assumed the chronic type extending over two weeks' time, after which the animal gradually became convalescent.

Hog 617, which received the Berkefeld-filtered blood from Hog 613 (the animal which developed the most acute form of the disease of the three cases described above), showed unmistakable signs of hog cholera about six days after inoculation. The course of the disease in this animal was of the chronic type and death occurred two months after inoculation.

Hog 621.



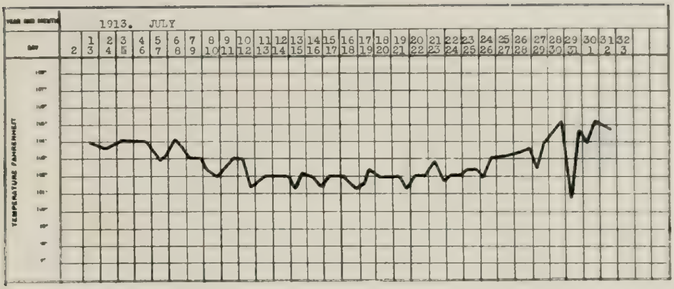
June 30. Intramuscular injection of 10 c.c. of blood of Hog 614, diluted 1:4, and Berkefeld filtered.
July 6-9. Animal not normal, appetite poor, general malaise.
July 10. Condition improved.
July 14. Condition normal.
July 25. Released as healthy immune.

Hog 621, inoculated with the Berkefeld-filtered serum of Hog 614, showed mild symptoms of cholera eight days after inoculation. After being slightly "off feed" for two or three days the animal regained its normal condition.

After the conclusion of this experiment, Hogs 620 and 621 were confined for several weeks in an infected enclosure with cholera pigs. No evidences of disease developed, thus establishing the fact that the animals possessed active immunity.

On July 1 one of the transfers, which was made from Strain 57, and which had been kept in the incubator since the time of inoculation, May 5, was submitted to dark field examination. It was found that the culture contained very few organisms which could be recognized as spirochetes, but many granules were present and the small amount of contamination consisted of a micrococcus. This culture, which had been incubated for a period of 57 days, was designated "Transfer 1-587-B," and two hogs,

HOG 622.



July 2. Intramuscular injection of 10 c.c. unfiltered suspension of positive culture No. 587 (May 5, 1913)—(July 2, 1913).

July 7-8. Appetite poor, general malaise, feces normal.

July 9. Apparently normal.

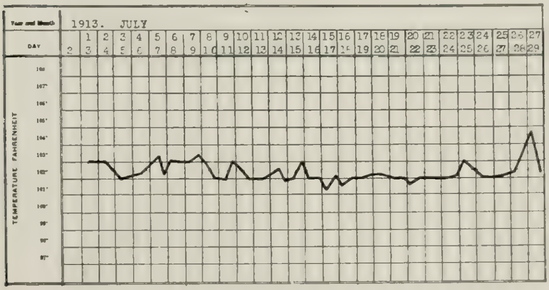
July 22. Exposed to hog cholera; placed with sick animals and injected intramuscularly with 2 c.c. of virus from Hog 631.

July 29-August 2. Symptoms of paralysis and chorea. Appetite fairly good. Animal not emaciated.

August 3. Animal found dead and examined.

Typical lesions of hog cholera absent; intestinal mucosa normal. Culture made from edematous fluid of muscular tissue shows *Str. pyogenes* present.

HOG 623.



July 2. Intramuscular injection of 10 c. c. unfiltered suspension of positive culture No. 587 (May 5, 1913)—(July 2, 1913).

July 7-8. Appetite poor, general malaise, feces normal.

July 9. Apparently normal.

July 22. Exposed to hog cholera; placed with sick animals and injected intramuscularly with 2 c.c. of virus from Hog 631.

July 29. Animal showed symptoms of paralysis and chorea. Could not walk.

July 30. Found dead and examined.
Mucous membrane of small and large intestine congested. No ulcers present in cecum.

Typical lesions of cholera absent.

Nos. 622 and 623, were each injected intramuscularly with 10 c.c. of the suspension of this culture in sterile water. Six days after the inoculation of these animals they showed, in addition to slight temperature changes, mild symptoms of illness. The appetite was poor and the animals were inclined to be listless and inactive. After about two days of inactivity and loss of appetite, they again resumed a normal condition and continued so until exposed to cholera.

On July 22 these animals were exposed to the disease by inoculation, natural exposure, and access to carcass of hog dead from cholera. On July 28 both hogs, Nos. 622 and 623, developed symptoms of paralysis and chorea. On July 29 Hog 623 was found dead and autopsy did not show lesions of hog cholera. Dark field examination of the mucosa of the cecum failed to show any spirochetes. Hog 622 on July 30 showed a moderate rise in temperature, but did not lose appetite or manifest other symptoms of cholera. The right front leg became enormously swollen. On August 3 Hog 622 was found dead. No characteristic lesions of hog cholera were present and cultures made from the serous fluid in the swollen leg developed *Str. pyogenes*. Several preparations were made from the mucosa of the cecum and were submitted to dark field examination with negative results.

On account of the absence of typical symptoms and lesions of hog cholera, also the course of the disease in the control animal (see notes below on Hog 641) and the presence of *Str pyogenes* in the edematous tissue of Hog 622, it seemed probable that Hogs 622 and 623 died from infection other than hog cholera.

III. Transfer 2. Third Generation on Artificial Media.

On May 27 a number of transfers were made from Culture Transfer 1-587-A. These cultures were incubated under anaerobic conditions from May 27 to July 2, when they were removed from the incubator and examined by the dark field method. One of these transfers, designated "Transfer 2-587-A," contained very little contamination represented by one species of micrococcus, and while only a few spirochetes could be found scattered through the medium, the culture contained many granules. This culture, Transfer 2-587-A, was macerated in sterile water, and on July 3 Hogs 627 and 628 were each inoculated intramuscularly with 10 c.c. of the suspension of this transfer. Five days after inoculation both of these animals showed indications of indisposition. From July 8 to July 12, the appetite was poor and the animals were inactive. On July 14 the condition of both hogs was much improved, and on July 16 both were pronounced apparently healthy.

On July 22 these animals were exposed to hog cholera in the same manner as Hogs 622 and 623, described above. After seven days' incubation both animals developed symptoms of cholera. Hog 627 succumbed to the disease 22 days after exposure. Hog 628, after suffering from the disease in chronic form, gradually became convalescent and on September 1 was released. Both animals showed greater resistance and maintained a better clinical condition than did the control Hog 641.

On July 11, during the period in which Hog 628 was showing a general reaction from the injection of Transfer 2-587-A, a few cubic centimeters of blood were drawn from the tail, defibrinated, diluted 1:4 in sterile water, and filtered through a bacteria-proof Berkefeld. On July 15 Hog 635 received intramuscularly 5 c.c. of the Berkefeld filtered serum, diluted 1:4, of Hog 628. This animal showed mild symptoms five days after inoculation and soon developed hog cholera of the subacute type. Four weeks after inoculation Hog 635 was found dead. Autopsy revealed typical lesions.

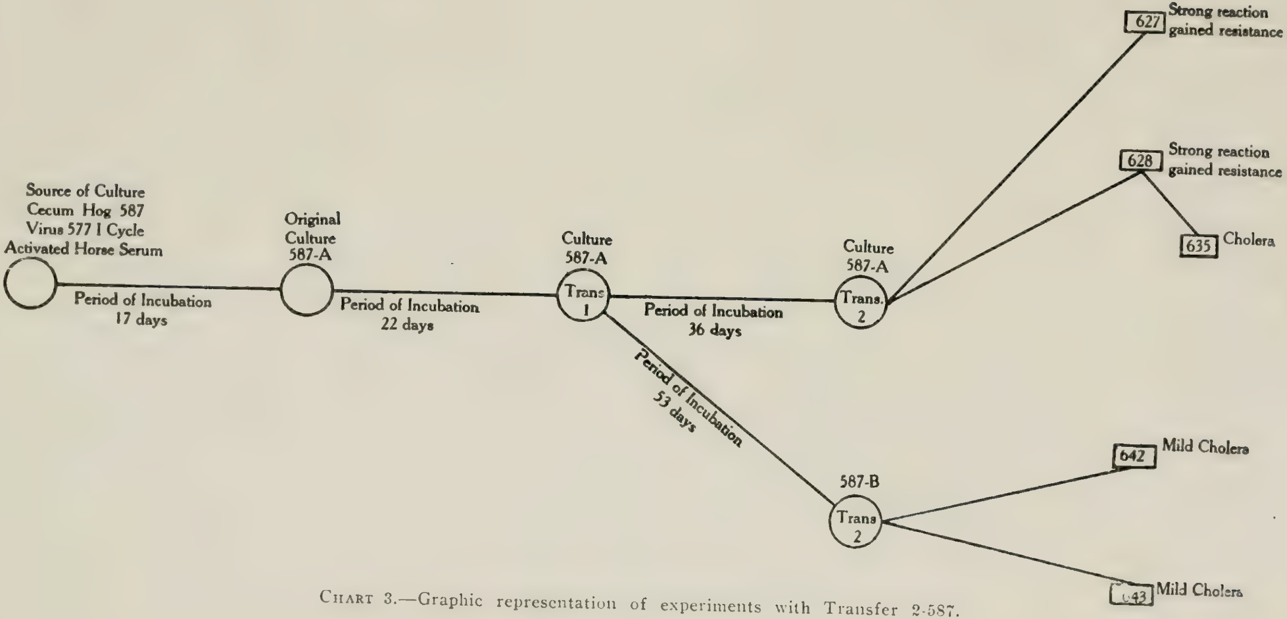
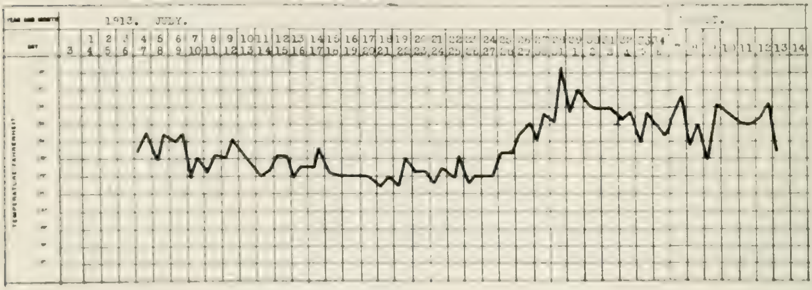


Chart 3.—Graphic representation of experiments with Transfer 2-587.

On July 24 Hogs 642 and 643 each received intramuscularly 5 c.c. of suspension in physiologic salt solution of Culture Transfer 5-587-B. Eight days after inoculation both animals manifested symptoms of mild cholera. They continued to show inactivity, anorexia, arched back, mild blepharitis, and roughened coat for a period of five days, after which they apparently regained a normal condition, which was followed by a recurrence in the case of both Hogs 642 and 643, in seven and twelve days, respectively. Both cases terminated fatally.

Hog 627.



July 3.	Intramuscular injection of 10 c.c. unfiltered suspension culture No. 587 (May 27, 1913)—(July 3, 1913).
July 8-12.	Poor appetite, inactive, eyes dull.
July 12.	Nervous symptoms.
July 14.	Condition improved.
July 16.	Animal apparently normal.
July 22.	Intramuscular injection 2 c.c. Virus Strain 631.
July 23.	Had been fighting with strange hogs.
July 29.	Animal suffering from a relapse, increasing emaciation; symptoms of acute type of cholera.
August 13.	Animal dead.

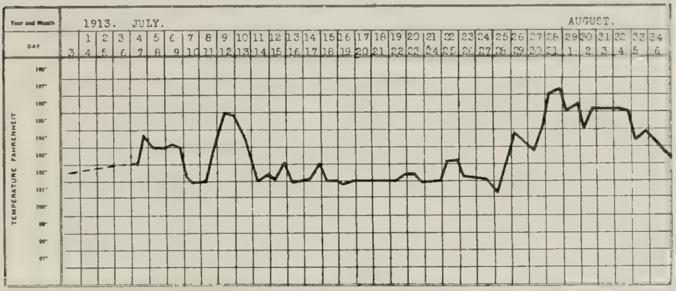
Typical lesions of hog cholera, subcutaneous tissue, lungs, kidney, lymphatic glands, cecum.

IV. Filtered Cultures.

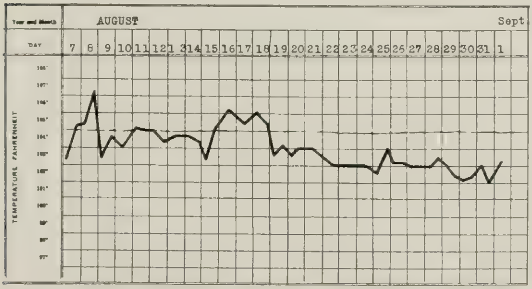
(a) Transfer 1.—Transfer 1-587-B, described above, a suspension of which was inoculated into Hogs 622 and 623, was also utilized in filtration experiments, which were first suggested by our colleague, Dr. N. S. Ferry. This culture, which had been incubated for a period of 57 days, after being macerated in sterile water in the proportion of one part culture to ten parts water, and thoroughly shaken for about one hour, was passed through a Berkefeld filter. The filter used was a bacteria-proof Berkefeld filter, pressure less than one atmosphere, time of filtration about 20 minutes, and volume of filtrate about 15 c.c. Cultures from this filtrate were made, both aerobically and anaerobically, and no bacterial growth resulted. Dark field examination of the filtrate revealed only relatively few granules. Hogs 624 and 625 on July 3 each received intramuscularly 10 c.c. of filtered culture, Transfer 1-587-B, diluted 1:10 in sterile water. Five days after inoculation both of these animals gave evidences of illness. From July 8 to July 10, the appetite was poor, eyes dull, and animals listless. On July 11 the condition of both hogs was improved, and on July 16 they were apparently healthy.

These hogs, Nos. 624 and 625, were exposed with control Hog 641 (see p. 578), by inoculation and by natural exposure. Three days after exposure Hog 624 showed evidences of illness, apparently due to gastritis and autointoxication, resulting from the ingestion of pus from an infected carcass. After a few days this animal fully recovered and exhibited a thrifty condition throughout the experiment, thus proving the existence of active immunity. Hog 625 showed scarcely any reaction as the result of the exposure, and was considered as being actively immunized by the filtered Culture Transfer 1-587-B.

Hog 628.



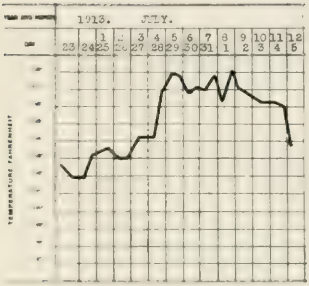
Hog 628 (*continued*).



July 3.	Intramuscular injection of 10 cc. unfiltered suspension culture No. 587 (May 27, 1913)—(July 3, 1913).
July 8-13.	Inactive, anorexia.
July 15.	Animal apparently normal.
July 22.	Intramuscular injection 2 c.c. Virus Strain 631.
July 30-August 5.	Symptoms of chronic type of cholera.
August 6.	Emaciated and growing progressively weaker.
September 1.	Animal normal, recovered and released.

On July 9, during the reaction following inoculation with filtered culture, a specimen of blood taken from the tail of Hog 625 was defibrinated, diluted 1:4 in sterile water, and filtered through the Berkefeld filter. On July 15 Hog 634 received intramuscularly 5 c.c. of the diluted, filtered blood from Hog 625. Five days after inoculation of Hog 634

Hog 641.



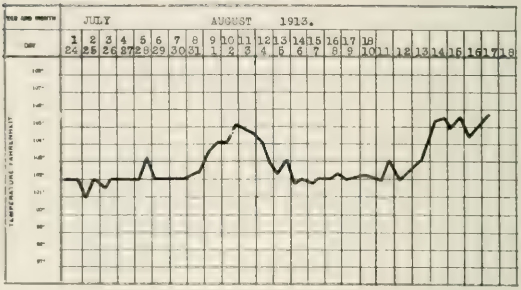
July 24. Intramuscular injection of 2 c.c. virus.

July 29. Typical symptoms of hog cholera.

August 5. Animal died.

Lesions: lungs, areas of hepatization; spleen, enlarged, soft and friable, engorged with blood; kidneys, petechiated; lymphatic and inguinal glands, enlarged and hemorrhagic; cecum, mucosa congested, small ulcers near ileocecal valve. *Spirochæta suis* present.

Hog 642.



July 24. Intramuscular injection 5 c.c. physiologic salt suspension Culture 587 (May 27, 1913)—(July 20, 1913), Berkefeld filtered.

August 1. Symptoms of anorexia; malaise, slight blepharitis.

August 5. Animal normal.

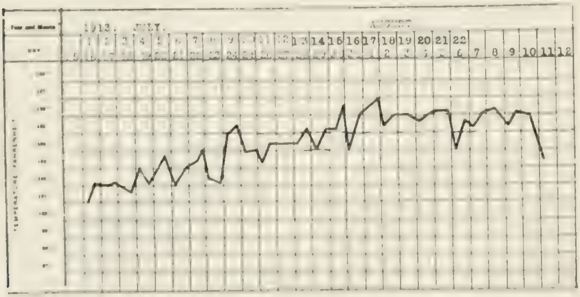
August 10. Temperature normal, though the appearance was not normal.

August 14. Indication of relapse; typical symptoms of cholera.

August 18. Animal found dead.

Typical lesions of hog cholera; subcutaneous tissue, heart, lungs, spleen, kidney, lymphatic system, cecum; *Spirochæta suis* present.

Hog 635.



July 15. Intramuscular injection of 5 c.c. blood serum of Hog 628, diluted 1:4, and Berkefeld filtered.

July 20-22. Animal "off feed," dull, inactive.

July 24. Condition improved; appetite poor.

July 29-August 5. Symptoms of subacute type of cholera.

August 6. Nearly moribund.

August 11. Animal died.

Typical lesions of hog cholera: lungs, heart, spleen, kidney, cecum, and lymph glands.

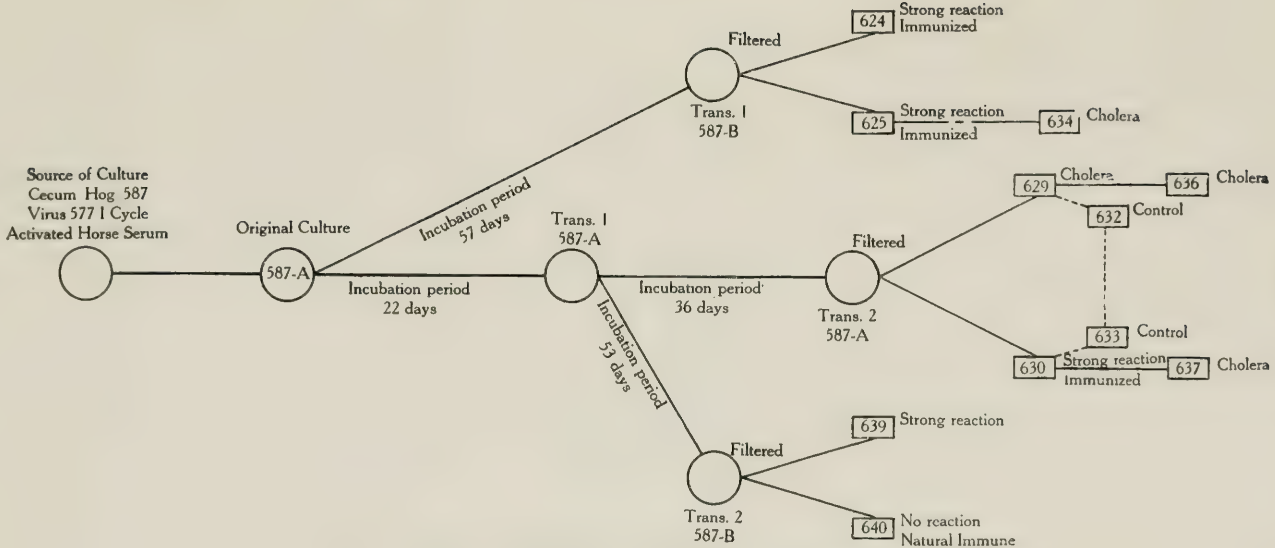
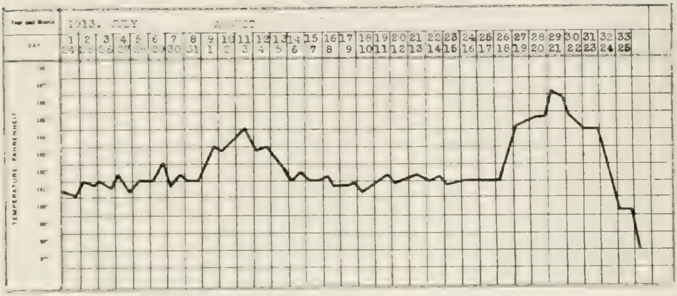


CHART 4.—Graphic representation of experiments with filtered cultures.

Hog 643.



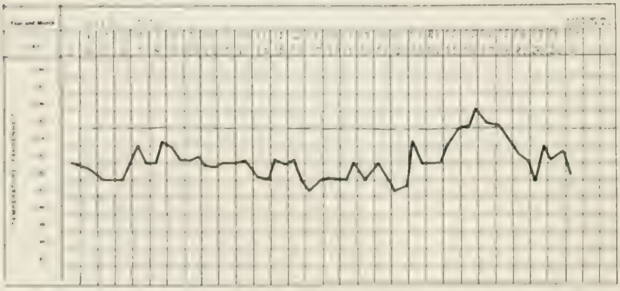
July 24. Intramuscular injection 5 c.c. physiologic salt suspension Culture 587 (May 27, 1913)—(July 20, 1913), Berkefeld filtered.
August 1. Symptoms of anorexia, malaise, slight blepharitis.
August 5. Animal normal.
August 10-17. Animal apparently normal.
August 23. Typical case of hog cholera.
August 26. Animal found dead.

Typical lesions of hog cholera: heart, lungs, spleen, kidney, lymphatic glands, subcutaneous tissue, and cecum.

the temperature rose two degrees, the animal was "off feed" and became constipated. This animal developed cholera of the subacute type and was found dead about four weeks after inoculation.

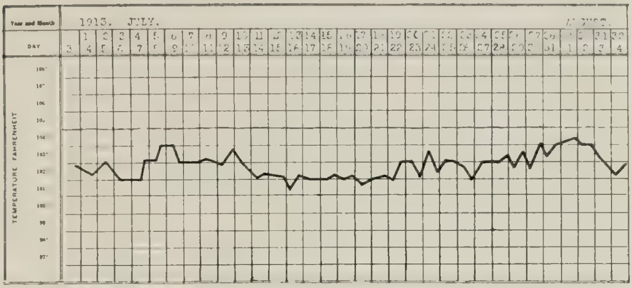
(*b*) *Transfer 2.*—Culture Transfer 2-587-A, described above, on July 2 was filtered and treated in exactly the same manner as described concerning the Berkefeld filtration of Culture Transfer 1-587-B. Transfer 2-587-A had been grown in the incubator for 36 days. On July 3 Hogs 629 and 630 were each injected intramuscularly with 10 c.c. of Culture Transfer 2-587-A, diluted 1:10, and filtered through bacteria-proof Berke-

Hog 624.



July 3. Intramuscular injection of 10 cc. Culture 587 (May 5, 1913)—(July 2, 1913), diluted 1:10, and Berkefeld filtered.
July 8-9. Anorexia, dull, listless.
July 10. Condition improved.
July 13. Animal apparently normal.
July 22. Intramuscular injection 2 c.c. Virus Strain 631.
July 23. Animal had been fighting with strange hogs.
August 1. Animal normal. Released.

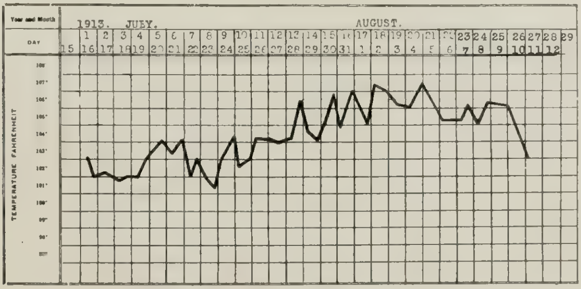
Hog 625.



July 3. Intramuscular injection of 10 c.c. Culture 587 (May 5, 1913)—(July 2, 1913), diluted 1:10, and Berkefeld filtered.
July 8-9. Poor appetite, eyes dull, inactive.
July 14. Animal apparently normal.
July 22. Intramuscular injection 2 c.c. Virus Strain 631.
August 4. Animal released.

feld. About eight days after inoculation both animals showed unmistakable symptoms of hog cholera. Hog 630, however, exhibited a milder case than Hog 629. Eleven days after inoculation Hog 630 quickly regained normal condition, rise in temperature subsided by crisis, and on July 17 the animal was pronounced normal. Subsequent exposure with control Hog 641 (see p. 482) demonstrated a condition of active immunity. Hog 629 developed a subacute case of hog cholera. The course of this disease lasted for weeks. This animal manifested typical symptoms of the disease, and after July 12 the more important symptoms

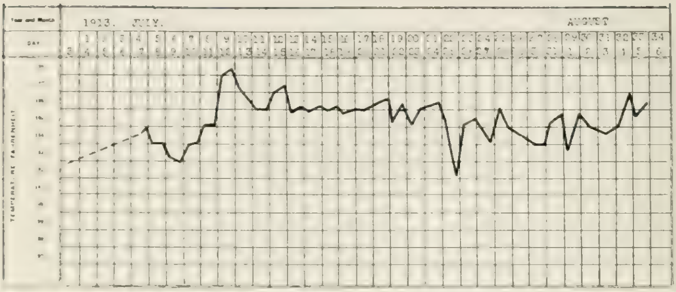
Hog 634.



July 15. Intramuscular injection of 5 c.c. blood serum of Hog 625, diluted 1:4, and Berkefeld filtered.
July 20-21. Inactive, poor appetite, somewhat constipated.
July 24. Apparently normal.
July 27-August 5. Symptoms of subacute type of cholera.
August 6. Nearly moribund.
August 12. Animal found dead.

Typical lesions of hog cholera in subcutaneous tissue; heart, lungs, kidney, spleen, cecum, and lymph glands.

Hog 629.



July 3. Intramuscular injection 10 c.c. filtered Culture 587 (May 27, 1913)—(July 3, 1913), 1:10, Berkefeld filtered.

July 9. Appetite poor.

July 10-20. Symptoms of subacute type of cholera: anorexia, blepharitis, malaise, roughened coat, emaciation, and profuse diarrhea.

July 24. Improvement noted.

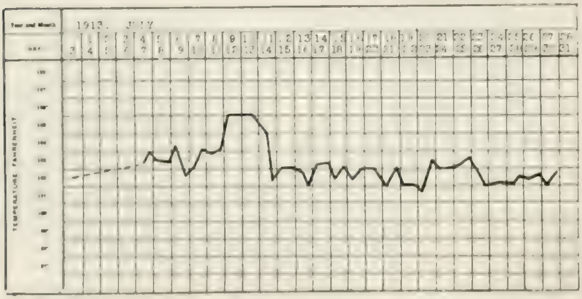
July 28. Relapse.

August 6. Nearly moribund.

August 7. Animal found dead.

Lesions: heart, ecchymosis on auricles; lungs, complete consolidation; spleen, enlarged, soft, friable; hemorrhagic; kidney, few petechiæ; cecum, ulcers, near ileocecal valve; inguinal and other lymph glands, enlarged, hemorrhagic; adhesions of ascending colon to peritoneum; extravasation in subcutaneous tissue. *Spirochæta suis* present.

Hog 630.



July 3. Intramuscular injection of 10 c.c. filtered Culture 587 (May 27, 1913)—(July 3, 1913), 1:10, Berkefeld filtered.

July 8. Poor appetite.

July 9-14. General inactivity, poor appetite, no apparent emaciation or loss of general tone.

July 15. Appetite good.

July 17. Apparently normal.

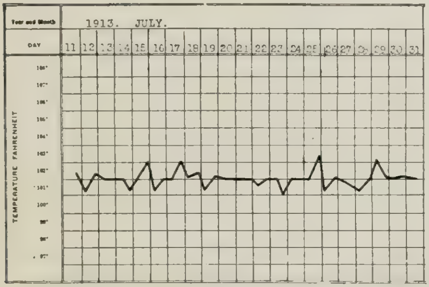
July 31. Animal normal. Released.

noted were anorexia, malaise, blepharitis, emaciation, and roughened coat. Hog 629 was found dead on August 7, 35 days after inoculation.

As a control on this experiment, Hogs 629 and 630, on July 11, were transferred from the room which they had occupied in the experimental stable to another, and after disinfection of the room normal Hogs 632 and 633 were immediately placed in the quarters formerly occupied by sick Hogs 629 and 630. An examination of the dates on the clinical charts will show that this rearrangement was made during the height of the disease in Hogs 629 and 630. Hogs 632 and 633 were subjected to exactly the same conditions as those attending the care and treatment of Hogs 629 and 630, except, of course, that they received no inoculation with infectious material. These control animals remained perfectly normal in every way, and after being kept as controls for some time were utilized in experiments.

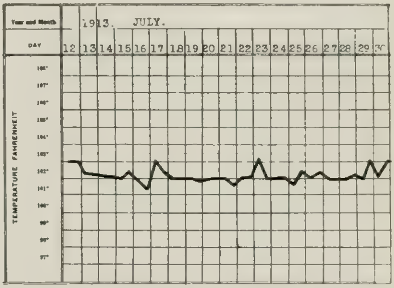
Hogs 636 and 637 on July 15 each received an intramuscular injection of 5 c.c. of the defibrinated, diluted, and Berkefeld-filtered blood serum of Hogs 629 and 630, respectively. Both of these animals developed typical symptoms of hog cholera and both were moribund 26 days after inoculation. Practically the only difference observed in their clinical appearance was that Hog 636, which had received the filtered blood from Hog 629, showed more severe symptoms of the disease after a shorter incubation period than those manifested in Hog 637, which had been inoculated with the blood from Hog 630. This result was consistent, as the injection of the filtered culture described above into Hog 629 produced the subacute type of hog cholera, while Hog 630, after a marked reaction, quickly regained normal condition.

HOG 632.



Normal hog.

HOG 633.



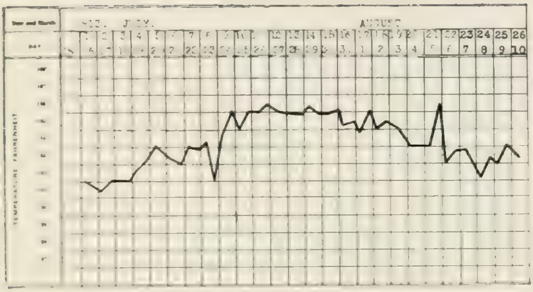
Normal hog.

In order to make the present experiment more complete Hogs 639 and 640 were injected each intramuscularly with 4 c.c. of suspension of Culture Transfer 2-587-B, which, after 53 days' incubation, had been macerated in sterile water, diluted 1:6, and subjected to filtration through the Berkefeld.

From the fifth to the ninth day following inoculation Hog 639 showed a marked reaction. During this period the animal was somewhat inactive and had poor appetite. Nine days after inoculation Hog 639 regained normal condition.

Hog 640 manifested no apparent reaction from the inoculation. This was regarded as an indication of natural immunity, as Hog 640 is a large brood sow and is probably a "natural immune."

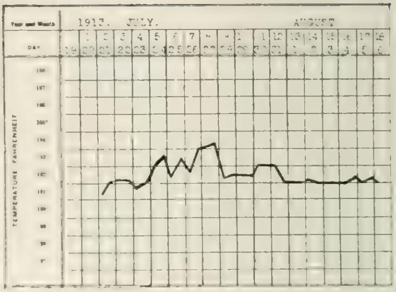
Hog 636.



July 15. Intramuscular injection of 5 c.c. blood serum of Hog 629, diluted 1:4, and Berkefeld filtered.
July 21. Animal "off feed" and constipated.
July 22-August 5. Typical symptoms of subacute type of cholera.
August 6. Animal nearly moribund.
August 11. Animal killed.

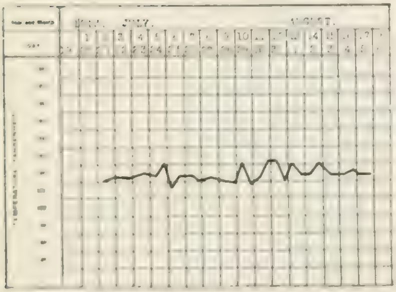
Typical lesions of hog cholera in lungs, spleen, kidney, cecum, and lymph glands.

Hog 639.



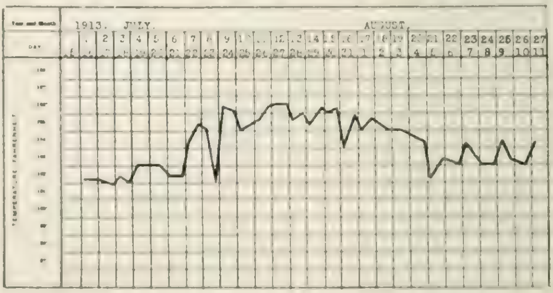
July 19. Intramuscular injection of 4 c.c. suspension Culture 587 (May 27, 1913), diluted 1:6, and Berkefeld filtered.
July 24-28. Anorexia, inactivity.
July 29. Normal.

Hog 640.



July 19. Intramuscular injection of 4 c.c. suspension Culture 587 (May 27, 1913), diluted 1:6, and Berkefeld filtered.

Hog 637.



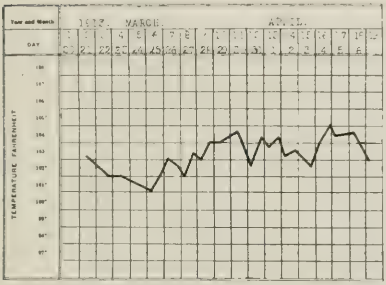
July 15. Intramuscular injection of 5 c.c. blood serum of Hog 630, diluted 1:4, and Berkefeld filtered.
July 21. Appetite poor; constipated.
July 22. Anorexia, constipation and diarrhea, weakened condition, emaciation.
August 6. Animal nearly moribund.
August 12. Animal killed.

Typical lesions of hog cholera; lungs, kidney, lymph glands, mucosa of cecum congested.

V. UNSUCCESSFUL INOCULATIONS WITH CULTURES, CONTROL ON POSITIVE RESULTS.

(*a*) *Strain 576.*—Hog 576 on March 20 was inoculated intramuscularly with 5 c.c. of hog cholera virus, strain Kansas III. This animal developed typical symptoms of cholera, and being moribund 18 days after inoculation, was bled and examined. The ordinary lesions of hog cholera were present and the material from the ulcers in the cecum, when examined by the dark field method, showed numerous spirochetes and spirilla of various forms. From this material in the ulcers cultures were made, and after three days' incubation some of these cultures were submitted to dark field examination. On account of the fact that some large forms of spirochetes were present, which at that time were regarded as possibly the same form which had repeatedly been found in the blood of cholera hogs, Culture 576-A was macerated in physiologic salt solution and 5 c.c. inoculated intravenously into Hog 584.

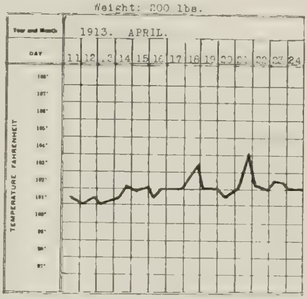
HOG 576.



March 20. Intramuscular injection of 5 c.c. Kansas III virus.
March 29. Animal not eating, general symptoms appearing.
April 4. Animal weak.
April 8. Moribund, bled from the carotid artery.

Typical lesions: lungs, heart, spleen, lymph glands; ulcers in cecum.

HOG 584.



April 11. Intravenous injection of 5 c.c. physiologic salt suspension, Culture 576.

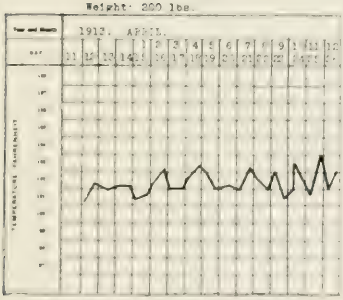
Hog 584, which received Culture 576-A, showed a slight thermal reaction on April 18, seven days after injection with the culture. Again, on April 21, the temperature rose two degrees. These thermal reactions were possibly due to the presence of a variety of contaminating organisms in the suspension, as no clinical symptoms were apparent in the animal. On April 25 the animal was released as normal and subsequent inoculation with hog cholera serum showed that Hog 584 was susceptible to the disease.

From the original culture, 576-A, transfers were made on April 11 and incubated under anaerobic conditions. Transfer 1-576-A, six days after inoculation, on being submitted to dark field examination, showed the presence of relatively large spirochetes, similar to those which had been found in the original culture, 576-A. Therefore, Transfer 1-576-A was suspended in physiologic salt solution and 2.5 c.c. of the suspension injected intravenously into Hog 585 on April 14. Five days after inoculation the animal showed an irregular temperature ranging between 102°

and 103°, and this variation in thermal condition was present for several days following. However, no symptoms of hog cholera were apparent and it was concluded that the fluctuating temperature was possibly due to the injection of contaminating organisms which were present in Transfer 1-576-A. The animal was finally released, and on being injected later with horse serum virus, hog cholera was produced and death eventually resulted.

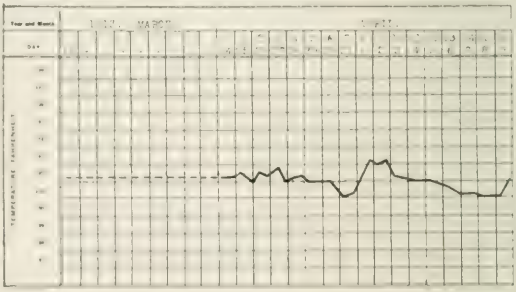
(*b*) *Strain 560*.—On February 14 Hog 560 received 5 c.c. of Kansas III virus intramuscularly. Six days after inoculation the animal developed symptoms of acute cholera, and after a duration of the disease for five days, being moribund, was bled and examined. Typical lesions of cholera were found on autopsy. Numerous spirochetes of various forms were found in the necrotic material contained in the ulcers of the cecum. Inoculations into culture media were made in the usual way, and after five days' incubation one of these cultures, designated as "Culture 560-A," after being examined on the dark field, was found to contain a growth of spirochetes. Contamination with various forms of bacteria and cocci, however, was present. In an attempt to eliminate as much of this contamination as possible, Culture 560-A was transferred into various culture media containing different chemicals, for the purpose of exerting slight germicidal action and the possible inhibition of the extraneous organisms. On March 24, after 21 days' incubation, Transfer 1-560-A, which had been made in a horse serum medium containing a small percentage of formal, was found, on dark field examination, to contain a few spirochetes, but no granules were observed. Two cubic centimeters of a physiologic salt suspension of Transfer 1-560-A were inoculated intravenously, March 24, into Hog 571. This animal showed no apparent result following the inoculation of this culture, except for a slight reaction which occurred during the eighth and ninth days after the inoculation. Subsequent injection of Hog 571 with horse serum virus resulted in the death of the animal from hog cholera of the acute type.

Hog 585.



April 14.	Intravenous injection of 2.5 c.c. physiological salt suspension, Transfer 1, Culture 576.

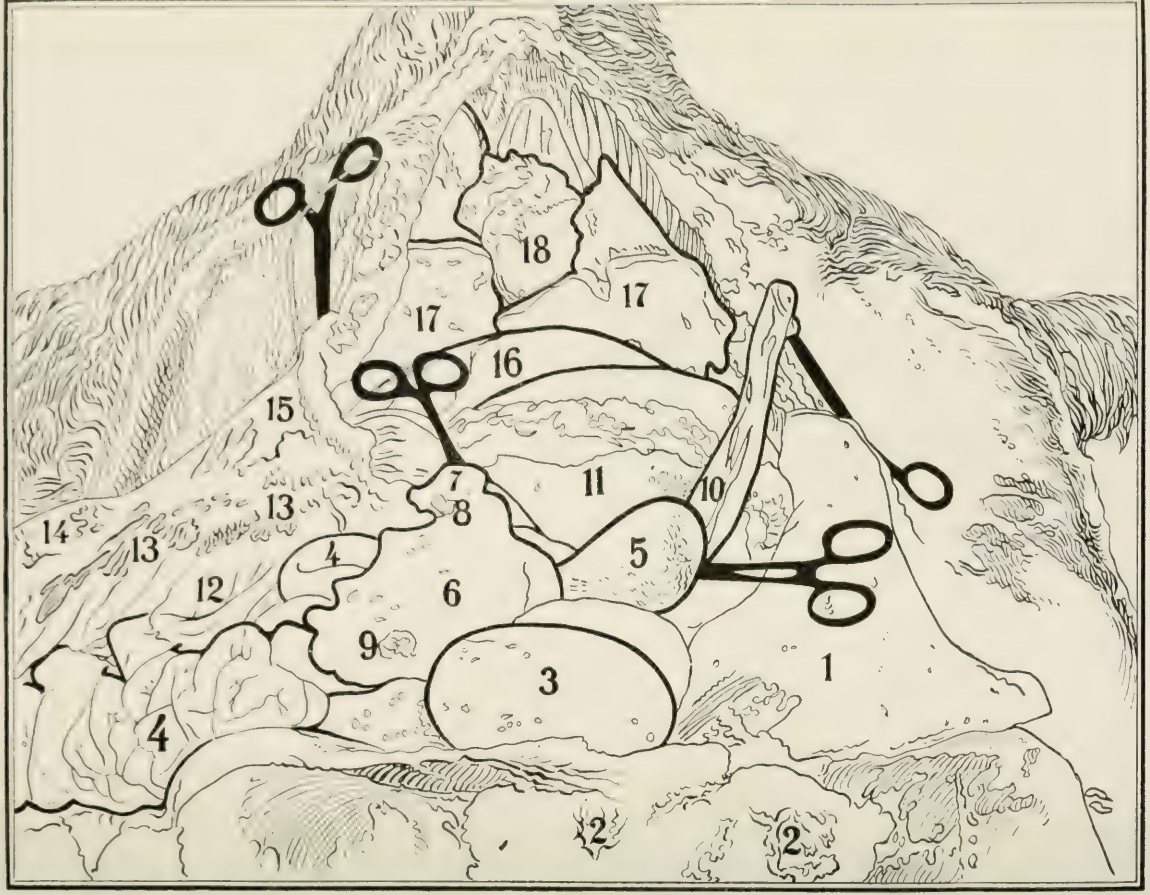
Hog 571.



March 15.	Blood normal.
March 15-24.	Animal normal.
March 24.	Intravenous injection of 2 c.c. physiological salt suspension, Culture 1, Transfer 2, Formol (March 3, 1913), Hog 560.
March 31.	Animal normal.
April 3.	Animal a little "off feed."

This includes all of those experiments which proved unsuccessful, and serve as admirable controls on the results given. It will be noticed that the unsuccessful experiments included under the last heading were conducted during the months of March and April. At that time it had not been definitely determined that the crypts of the ceca of normal hogs may contain spirochetes and spirilla. In so far as our observations go, these organisms are usually relatively large and altogether unlikely to be related to the form which we have designated as *Spirochæta suis,* the latter being found in the intestinal ulcers and the external local lesions of cholera hogs. No doubt, the small spirochete described in the first part of this paper as *Spirochæta suis* was present in the ulcers of Hogs 576 and 560, this material being used in the inoculation of the cultures described under the present heading. However, all of the inoculations into hogs, of cultures from these two strains, were made after a comparatively short period of incubation. This explains the failure to produce positive symptoms of hog cholera with the cultures, as our later results reported above indicate that it is necessary to incubate the cultures for several weeks in order to grow *Spirochætæ suis.*

The most important bearing, however, of these unsuccessful attempts to produce the disease with the cultures noted under the last heading pertains to the control which they give on the results described in the preceding pages. The foregoing positive results from inoculations of filtered and unfiltered cultures of *Spirochæta suis* are lacking in one detail. Without some means of controlling the results, it might be logically assumed that those animals which had received hog cholera from the injection of cultures and transfers did so as the result of the injection of a small amount of original virus, which possibly might have been transferred through the cultures. This we know to be untrue because of the failure to produce hog cholera in animals inoculated with cultures and transfers from Strains 576 and 560. Positive results followed the inoculation of Hog 559 with the original culture, 551-A, which had an incubation period of only five days. The results may have been due to some of the original virus having been transferred to the animal through culture. However, we believe that the succeeding results are sufficiently controlled by the



1, Peritoneum (showing small blood extravasations). 2, 2, Inguinal Glands (enlarged and hemorrhagic). 3, Bladder (showing small blood extravasations). 4, Small Intestine (Ileum) (covered with punctiform hemorrhages). 5, Kidney (petechiated). 6, Cecum (ental surface showing excessively congested mucosa). 7, Ileocecal Valve. 8, 9, Typical button ulcers in cecum. 10, Spleen (enlarged, soft and gorged with blood). 11, Stomach. 12, Mesentery (showing minute hemorrhagic spots). 13, 13, Mesenteric Glands (enlarged and excessively hemorrhagic). 14, 15, Small Intestine (Jejunum) (containing ulcerated mucosa and extensive hemorrhage). 16, Liver. 17, 17, Lungs (showing areas of congestion facts that Hog 584 failed to develop hog cholera after inoculation with Culture 576-A, and that Hogs 571 and 585 did not respond to the injection of Transfers 1-576-A and 1-560-A, respectively.

CONCLUSIONS.

Spirochæta suis is an organism found in the intestinal ulcers, crypts in the ceca, and external local lesions of animals suffering from hog cholera. It is a typical spirochete, simulating in many characteristics *Spirochæta pallida, Spirochæta gallindrum,* and other forms whose morphology and life history are becoming better understood. *Spirochæta suis* appears to be capable of breaking up into granules, and these granules may play an important part in the life cycle and physiological functions of the organism. They are present in the blood of cholera hogs in cultures of *Spirochæta suis,* and are capable of producing the disease in healthy hogs.

In the blood of hogs suffering from cholera the presence of a relatively large spirochete in few numbers has been recognized As this organism has not been found in the blood of normal hogs it may represent *Spirochæta suis* in a transitional form. Its morphological variation from *Spirochæta suis,* as found in ulcers and local foci, may be due to the unfavorable action of blood as a medium or to its natural processes as a part of the life cycle of the species.

Spirochæta suis is an obligatory anaerobic organism and usually requires several weeks' incubation for growth to take place on artificial culture medium. It may be transferred from generation to generation on artificial culture medium. Cultures containing the organism in the form of granules and spirochetes may be passed through bacteria-proof filters and the spirochetes removed, the few small granules which pass through being capable of producing hog cholera or resistance to the disease.

Spirochæta suis is capable of producing typical hog cholera when injected into healthy hogs. This is true not alone of contaminated cultures made directly from the intestinal ulcers of cholera hogs; second and third generations on artificial culture media, containing the *Spirochæta suis,* as well as the Berkefeld filtrates of the same transfers, are capable of producing hog cholera and marked reactions, which confer more or less protection against the disease. The pathogenicity of these cultures does not appear to be due to the passage of an unknown "invisible microörganism" which is finally transmitted to healthy hogs by inoculation. Control experiments tend to show that the pathogenicity of the cultures of *Spirochæta suis* is due to the species itself in the form of spirochetes or granules.

Finally, in those hogs which receive the disease from cultures of *Spirochæta suis,* the organism is present in the intestinal lesions or local external lesions, as demonstrated by the dark field examination.

From the above results, which have practically fulfilled Koch's laws, in so far as it is possible with an organism possessing the biological characteristics of spirochetes, it may logically be concluded that *Spirochæta suis* is more nearly established as the specific cause of hog cholera than any other known organism.

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